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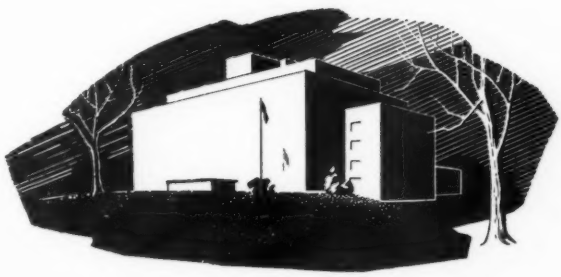
A Symposium on

Some Infectious Diseases

of Laboratory Rodents

Armed Forces Institute of Pathology



Foreword

Laboratory animals are subject to a wide variety of diseases which exert their effects in several ways: some smoulder in the animal population, subtly warping the results of experimentation; others crop up in the midst of an experiment to negate or distort all results; still others sweep through a colony and wipe out most of its members. The recognition and control of such diseases are extremely important in any soundly based research program involving laboratory animals.

Knowledge gained from study of spontaneous disease in animals can be applied not only to avoidance or elimination of disease but also to the development of principles that are applicable to other species, including man. Thus, such diseases may serve as models which can be reproduced and studied under laboratory conditions. In every experiment it is vital to distinguish experimentally induced lesions from incidental ones, and to do so requires competent knowledge of the lesions that can appear spontaneously in a given species.

Contributions to knowledge of disease in laboratory animals have been made by many investigators who have been prompted to study a spontaneous disease by a disastrous experience that interrupted the course of an investigation on a totally unrelated subject. Some of these studies have been reported in the literature, but usually in such widely scattered journals that they are not readily available. Many investigators have had a single, sometimes bitter, experience with a spontaneous disease in laboratory animals, but few have had a broad and continuous experience. This emphasizes the importance of assembling and publishing all data.

A Postgraduate Course, Pathology of Diseases of Laboratory Animals, was initiated in 1954 at the Armed Forces Institute of Pathology, for the purpose of disseminating information to medical, dental, and veterinary research workers and stimulating study in this field. As expected, the assembling of interested scientists from governmental, educational, and industrial institutions, for a week-long course of study, had a catalytic effect upon work in this area. This course, with yearly additions of subject matter, has been conducted in each subsequent year. Certain lectures by outstanding authorities who contributed to the first and second AFIP Postgraduate Courses, in 1954–55, have been selected for publication in this issue of the *Journal of the National Cancer Institute*. The highest praise is due this effort to give wider dissemination to information on a subject that is vital to every laboratory investigation in which animals are used.

T. C. JONES
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Contents

Page

Epidemic Diarrheal Diseases of Suckling Mice. ALWIN M. PAPPENHEIMER, M.D., *Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts* 861

Lymphocytic Choriomeningitis. FRED D. MAURER, LT. COL., VC, *Armed Forces Institute of Pathology, Washington, D. C.* 867

Mouse Poliomyelitis or Theiler's Mouse Encephalomyelitis. FRED D. MAURER, LT. COL., VC, *Armed Forces Institute of Pathology, Washington, D. C.* 871

Mouse Pox (Infectious Ectromelia). LEON Z. SAUNDERS, DVM, *Brookhaven National Laboratory, Upton, Long Island, New York* 875

Pathology of Infection with the JHM Virus. ALWIN M. PAPPENHEIMER, M.D., *Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts* 879

Tyzzer's Disease. LEON Z. SAUNDERS, DVM, *Brookhaven National Laboratory, Upton, Long Island, New York* 893

Myositis in Guinea Pigs. LEON Z. SAUNDERS, DVM, *Brookhaven National Laboratory, Upton, Long Island, New York* 899

Salivary-Gland Virus Disease of Guinea Pigs. JAMES E. COOK, CAPTAIN, USAF (VC), *Armed Forces Institute of Pathology, Washington, D. C.* 905

Infection in Laboratory Animals with Pleuropneumonia-like Organisms. JOHN B. NELSON, M.D., *Rockefeller Institute for Medical Research, New York, New York* 911

Myocarditis and Pulmonary Arteritis Associated with the Presence of Rickettsia-like Bodies in Polymorphonuclear Leukocytes. ALWIN M. PAPPENHEIMER, M.D., *Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts* 921

Salmonellosis in Laboratory Animals. ROBERT T. HABERMANN, DVM, and FLETCHER P. WILLIAMS, JR., B.S., *National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland* 933

Bartonellosis. RICHARD A. GRIESEMER, 1ST LT., VC, *Armed Forces Institute of Pathology, Washington, D. C.* 949

Encephalitozoon Infection in Laboratory Animals. DONALD H. YOST, CAPTAIN, VC, *Biophysics Division, Chemical Warfare Laboratories, Army Chemical Center, Edgewood, Maryland* 957

Joint Diseases of Laboratory Animals. LEON SOKOLOFF, M.D., *Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland* 965

The Identification and Control of Helminths in Laboratory Animals. ROBERT T. HABERMANN, DVM, and FLETCHER P. WILLIAMS, JR., B.S., *National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland* ... 979

Epidemic Diarrheal Diseases of Suckling Mice [1]

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I shall preface this discussion with a few personal remarks which will explain my current interest in mouse pathology. After retiring from Columbia University and the Presbyterian Hospital, in 1945, I accepted an invitation from my old and cherished friend, the late Professor J. Howard Mueller, and we set up shop in his Department of Bacteriology and Immunology at the Harvard Medical School. I was given a room and a technical assistant, and, from the first, enjoyed the collaboration of my younger colleagues. Dr. Mueller's untimely death has been a sad blow to me personally, as well as to the science of microbiology.

When I first arrived at the laboratory, Dr. Mueller told me that his mouse colony was suffering from a form of infantile diarrhea, which was causing a heavy mortality and considerable economic loss not only in his stock but also in many other breeding colonies. He and Dr. Cheever were engaged in making a very thorough epidemiologic study of the disease and had made many interesting observations on the clinical behavior, the seasonal incidence, the morbidity and mortality rates, and so forth. The diarrheal signs appeared in sucklings between the 10th and 15th day of life and never after weaning. The diarrhea was marked by the passage of copious watery stools, which soiled the infected mouse; in mice that survived the first 4 days, fecal material, caked about the anus, often caused complete obstipation. The outcome varied in individual litters from 100 percent mortality to 100 percent recovery. Bacteria of the salmonella group, which had been regarded by Syverton and Olitzky in 1934 (*1*) as the causative agent in a diarrheal disease in the breeding colony of the Rockefeller Institute, was not recovered from these mice, and the ulcerative lesions of the intestine, which they described, were never seen in our mice. Obviously this was a different disease.

The conclusions reached by Mueller and Cheever from their epidemiologic study were that the disease was almost certainly infectious, that

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.
[2] Deceased.

it was difficult to eradicate, and that the susceptibility of first litters, in contrast to subsequent ones, was greater (*2*).

Unfortunately, the entire breeding stock was destroyed by accident in July, 1946. However, a very similar diarrheal disease reappeared in their new stock; but as we shall see, the two epidemics, though similar in their symptomatology, were associated with different pathologic findings.

Dr. John Enders had examined a few sections of intestine and noted the occurrence of intranuclear inclusions, and together we began a more systematic study (*3*).

We found no ulcerations or other inflammatory changes in the intestines. We could, however, readily demonstrate the occurrence of intranuclear inclusions in the superficial epithelial cells of the small intestine. They were of two types, which we have designated as spherical and granular. The two types rarely occurred in the same nucleus but were usually found in different nuclei, and transition forms were not apparent.

The spherical forms, which stained red with eosin and were intensely fuchsinophilic, ranged from 1 to 3μ in diameter. They were sharply contoured, refractile and dense, and usually surrounded by a clear halo. Occasionally they were sausage-shaped.

In the granular type, the nucleus became filled with a grayish-purple mass which crowded the chromatin against the nuclear membrane. Under oil immersion, the material appeared as a cloud of minute granules embedded in a matrix (figs. 1 and 2).

These inclusions first appeared on the 10th day, concomitantly with the onset of the diarrhea, and were never seen after the 16th day. Seventy-five percent of the diarrheal group showed inclusions, whereas the inclusions were found in only 2 of 36 mice (5.7%) that appeared uninfected but that may well have had the disease in unapparent form as they came from the same stock and room in which diarrhea was prevalent.

In animals experimentally infected, by feeding infected fecal material to healthy stock on the 3rd day, the inclusions again appeared on the 10th day, were present on the 11th, 12th, and 13th day, and then disappeared. The reason seemed to be that the inclusion-containing cells were rapidly desquamated into the intestinal lumen. This selective exfoliation of the affected cells was followed by rapid restoration to normal.

We were impressed in our study by the resemblance of the inclusions to those described by Hammon and Enders (*4*), by Lawrence, Syverton, Shaw, and Smith (*5*), and by Lucas and Riser (*6*), in the infectious panleukopenia of cats. Whether there is any relation between the two viruses remains to be investigated.

After the accidental destruction of the breeding colony in the summer of 1946, new stock from different sources was brought in and a new colony started. Diarrhea was known to be endemic in some of these strains and soon reappeared in the new colony. Again, careful epidemiologic studies were made by Cheever and Mueller (*7*), which brought

out strain differences in susceptibility and seasonal and litter differences.

With Dr. Cheever's collaboration, I again studied sections of intestines and, to my surprise, found not the intranuclear inclusions, which I have described, but cytoplasmic inclusions (*8*). These varied in size, were intensely fuchsinophilic, and were located above, below, or to one side of the nucleus. Occasionally, a single cell contained several inclusions, which were restricted to the superficial epithelial cells of the small intestine, and again were unaccompanied by inflammatory reaction (fig. 3). In the disease experimentally produced by feeding infectious material, inclusions of this type were found in 97 percent of the cells during the first 3 days of the disease; only rarely were they present later, and then often only in desquamated cells lying free in the lumen. They were not found in healthy stock of corresponding age. In short, there seemed to be a clear-cut relation between the diarrheal disease and these cytoplasmic inclusion bodies.

If we accept the occurrence of inclusions as evidence of viral etiology, then it would seem that there are at least two different agents responsible for this disease: one, characterized by the occurrence of *intranuclear* inclusions, the other, by *cytoplasmic* ones. Much remains to be investigated, and no one has as yet studied the virus in detail to determine particle size, morphology, immunologic relationship between different strains, and so forth. Until this has been done, we must leave the matter as it stands. The experimental transmission to healthy stock by bacteria-free filtrate and the occurrence of the characteristic inclusions at least make the viral etiology certain.

Before leaving the subject, I must refer briefly to two other observations, the significance of which is very obscure. Cytoplasmic inclusions of larger size, one to a cell, always supranuclear and rather easily decolorized, have been found in healthy mice at different ages, and appear to be totally unrelated to the diarrheal disease. Whether they are indicative of some other latent viral infection we do not know, but unless one is familiar with the two different types of cytoplasmic inclusions, they may give rise to some confusion.

Also, I should like to mention the presence of vast numbers of gram-positive coccoid bodies, often adhering in a fuzzy fringe to the surface of the epithelial cells. Attempts to cultivate these on the usual laboratory media were not successful, so they are not ordinary staphylococci. They were found at times in healthy mice but occurred with far greater frequency in the diarrheal animals. What effect, if any, they may have in relation to the disease, we do not know.

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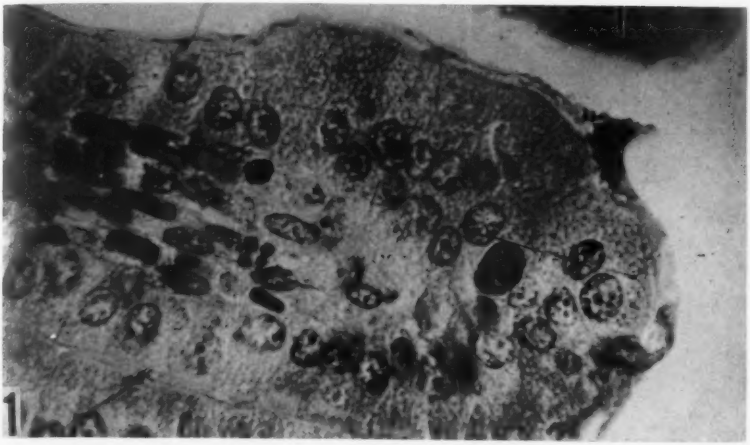
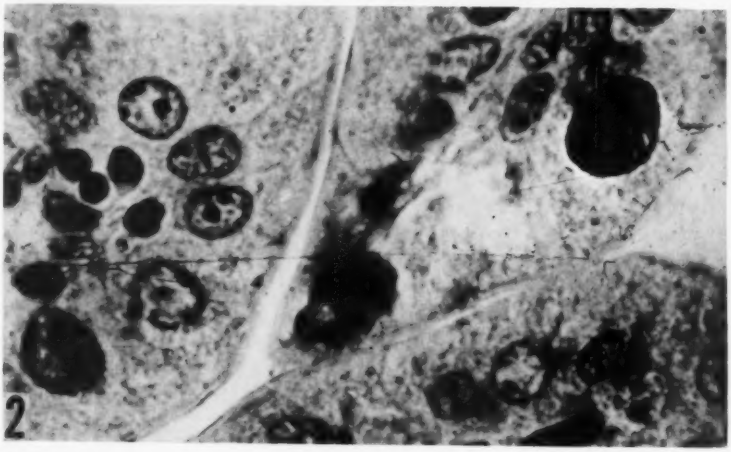
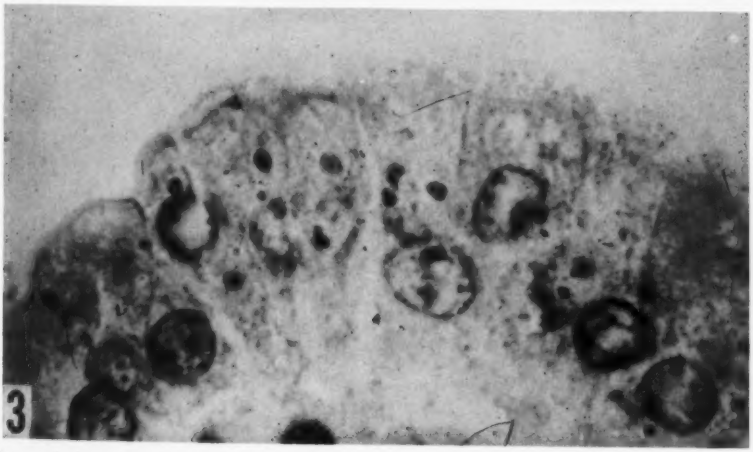
Plate 73

Figures 1 and 2.—Granular type of intranuclear inclusions. Margination of chromatin is evident in nuclei containing dark, granular bodies.

Figure 3.—Intracytoplasmic inclusions (dark, oval, and spherical bodies surrounded by clear zone).







Pappenheimer

459041—58——2

Lymphocytic Choriomeningitis [1]

FRED D. MAURER, *Lt. Col., VC, Armed Forces Institute of Pathology, Washington, D. C.*

Lymphocytic choriomeningitis is an acute infectious viral disease of man and animals, which in its rare but most acute form produces a fatal paralyzing meningoencephalitis. The virus is prevalent in mice which carry and transmit the disease, though 80 percent of them fail to show symptoms of the disease they harbor.

Host range.—The virus has been isolated from laboratory and house mice, monkeys, dogs, guinea pigs, roaches, ticks, and man. Experimental infection has been established in man, chimpanzees, monkeys, guinea pigs, rats, mice, dogs, and small pigs. In rabbits, young chicks, hamsters, and horses, the virus produces very slight, if any, symptoms but it has been recovered from blood or organs after a varying number of days (*1*, *2*).

Character of the virus.—Different strains of the virus vary in their pathogenicity, but there is no evidence of the existence of different antigenic types (*1*, *2*). The virus is from 33 to 60 mμ in size and is filtrable through the usual bacteria-retaining filters (*2*). It is killed by 55° C. in 20 minutes and rapidly inactivated by ultraviolet light. It has been cultivated on chick embryos and in tissue cultures (*2*). It stores well when frozen, when dried and held below 0° C., or when kept for some months in 50 percent neutral glycerin.

Transmission.—A high percentage of house mice are infected. In Washington, D. C., Armstrong *et al.* (*3*) found 21.5 percent to be infected. The virus has been found in dogs that also had distemper (*4*). Three fatal cases occurred in people working with canine virus vaccine (*5*). Virus has been isolated from ticks. Ticks carry the virus through their life cycle and nymphs have transmitted the disease to guinea pigs. Mosquitoes, *Aedes aegypti*, have transmitted the virus experimentally to guinea pigs. Monkey lice have transmitted the virus to other monkeys on two recorded occasions. Man is believed to be infected through the intact human skin, the conjunctiva, the respiratory tract, and the digestive tract. Man may be infected by blood-sucking insects (*2*). Laboratory workers have be-

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.

Journal of the National Cancer Institute, Vol. 20, No. 5, May 1958
459041—58——3

come infected from handling laboratory mice; other people from handling house mice (*6*).

Lymphocytic Choriomeningitis in Man

Four forms of infection are encountered in man: 1) an influenza-like disorder without central nervous system involvement; 2) a meningeal type, which may follow the influenza-type of infection; 3) a meningeal encephalitis, which may be fatal; and 4) an asymptomatic type from which immunity results. In general, recovery occurs within 2 or 3 weeks, and the disease is only rarely fatal (*1*, *2*, *7*).

Natural Lymphocytic Choriomeningitis Infections in Mice

Most colonies of laboratory mice have been found to harbor the virus unless special precautions have been taken. Of the infected mice, most carry the disease in a latent form and only about 20 percent show signs of infection and less than 2 percent die. It is not surprising to find the disease in most laboratory colonies in geographic areas where 10 to 20 percent of house mice have been found to be infected (*3*, *8*).

Signs of Infection

Only young mice are likely to show clinical illness; they are drowsy, emaciated, have roughened fur, and sit in a corner by themselves. Movement is slow and stiff and their legs appear long in proportion to their thin bodies. Paralysis does not occur. Signs of infection may be meager; of 5 mice showing only photophobia and conjunctivitis, 2 were positive (*8*). Tremors and spastic convulsions, which characterize the experimental disease in intracerebrally injected mice, have not been seen in cases of the natural disease. Congenital infections occur (*8*).

The gross lesions in mice are few, but pleural exudate, fatty liver, and an enlarged spleen are suggestive of lymphocytic choriomeningitis. Microscopically, there is leukocytic infiltration of the meninges in natural cases but usually not so severe as in experimental cases following intracerebral injections of the virus. The lungs show peribronchiolar and perivascular infiltrations with round cells and a slight thickening of of alveolar walls. The liver presents small collections of round cells near blood vessels, and scattered lymphocytes in interstitial tissue. Patchy reticuloendothelial hyperplasia occurs in the spleen.

Detection of the Disease

When there is virus elsewhere in the body, the intracerebral injection of sterile inert material such as sterile broth will elicit an active infection of the central nervous system. The injection of such material will give rise, in from 3 to 13 days, to meningeal lesions and signs indistinguishable from those in mice injected intracerebrally with the virus. The symptoms

include a marked tremor of the hind legs, which passes into spastic convulsions when the animals are lifted by the tail.

The virus may be isolated from brain, blood, or spleen of infected animals by the intracerebral injection of mice or guinea pigs. Suspensions of tissues pooled from a group of animals are more likely to contain the virus. Suspect mice may be tested for immunity by injecting them intracerebrally with virus.

Experimental Lymphocytic Choriomeningitis in Mice

On the 6th or 7th day following intracerebral inoculation, mice show general malaise, sit quietly alone, and have roughened fur. When lifted by the tail, rapid leg motions followed by leg tremors and convulsions occur. When dropped during a convulsion the hind legs may be stiffiy extended, the tail rigid, and the back humped. They may drag themselves around by the front legs. They die in these characteristic seizures after 1 or 2 days of symptoms.

There are no consistent gross lesions produced by the intracerebral inoculations of mice with virus, though enlargement of the spleen is common. Microscopically, round-cell infiltration, predominantly lymphocytic, occurs in the meninges especially at the base of the brain, in the pia-arachnoid, the chorioid plexus, and in the perivascular lymph spaces of submeningeal and subependymal vessels.

Lymphocytic choriomeningitis in laboratory animals is obviously very undesirable. It is a hazard to human health and likely to invalidate the results of experiments. The maintenance of a mouse colony free of lymphocytic choriomeningitis requires that disease-free breeding stock be obtained and kept isolated from wild rodents and that subsequent addition of laboratory animals be avoided. Separate attendants should be available for the breeding colony. Feed should be obtained from a clean source and stored out of contact with all rodents. Since lice and ticks have been shown capable of transmitting lymphocytic choriomeningitis, the possibility of insect transmission should be avoided in animal quarters. Attendants should be on the alert for suspect cases among guinea pigs and monkeys as well as mice. Periodic checks for latent virus in mice are desirable unless there are ample intracerebrally inoculated controls being used in routine work to provide a means of detection.

It is well for all laboratory workers to keep in mind that the virus is capable of penetrating the intact skin and that the urine of infected mice contains the virus.

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Mouse Poliomyelitis or Theiler's Mouse Encephalomyelitis [1]

FRED D. MAURER, *Lt. Col., VC, Armed Forces Institute of Pathology, Washington, D. C.*

Theiler's mouse encephalomyelitis is a viral disease characterized by flaccid paralysis resulting from necrosis of the ganglionic cells of the anterior horn of the spinal cord. The virus is carried in the intestinal tract of most laboratory white mice. Its wide distribution and its ability to produce cord lesions like those of the poliomyelitis viruses have made it of considerable interest.

Distribution and host range.—The virus is found wherever white mice are raised. Gray mice and kangaroo rats raised near white mice also harbor the virus (*1*). Olitsky observed that guinea pigs, rabbits, and monkeys are resistant to intracerebral and intraperitoneal injection of Theiler's virus, and that neutralizing antibodies do not develop in the monkey.

Natural occurrence.—According to Theiler and Gard (*2*), the virus is present in the intestinal tract of about two thirds of apparently normal white mice examined at 6 weeks of age. Olitsky (*3*) observed that mice less than 12 days of age were free of the virus; those from 20 to 25 days old were irregularly infected; while at 30 days of age they were regularly positive. They generally remained positive until about 6 months of age; after that the virus usually could not be isolated. Virus of the highest titer, 10^{-3}, was found in the contents of the small intestine, and virus of lower titer in the wall. Virus has been recovered irregularly from the mesenteric lymph nodes but not from other tissues. When infected mice were isolated and given sterile water and feed, virus was still being passed after 53 days, which was as long as the test ran (*2*). In spite of the high incidence of carriers, the virus invades the central nervous system in only about 1 in 4,000 mice. These spontaneous infections of the central nervous system are seen only in young mice.

Properties of the virus.—The virus is very small, only 9 to 13 mμ in diameter (*4*). It is filtrable through Seitz and other bacteria-retaining filters. The virus is inactivated by exposure at 50 to 55° C. for 30 minutes (*3*). It is quickly inactivated by ethyl alcohol but withstands exposure to ether (*4*). The *p*H optimums are at *p*H 8.0 and 3.3 (*4*). The virus has been propagated in tissue culture using mouse embryo brain.

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.

It has also been grown in chick embryos on the chorio-allantoic membrane, yolk sac, and in the allantoic cavity (*5*).

The Disease in Mice

The incubation period is from 7 to 30 days before paralysis appears. The infection closely parallels poliomyelitis in human beings. A flaccid paralysis starting in one leg gradually spreads to the others, the hind legs usually being most severely involved. The afflicted mice appear quite normal except for the paralysis. They seem surprisingly bright and alert and continue to eat, though dragging one or more legs. The tail has never been observed to be involved. Mice may regain use of their legs after partial paralysis, but recovery is never complete (*1*, *6*).

Lesions.—Atrophy of muscles is the only grossly apparent pathologic change. Microscopic study shows that the paralysis results from necrosis of the ganglionic cells of the anterior horn. Degeneration of these cells has been observed before the onset of paralysis. The necrosis is acute and is followed by neuronophagia. There may also be some focal and perivascular infiltration in the central nervous system (*1*, *6*).

Olitsky (*3*) describes the pathologic changes as neuronal necrosis, neuronophagia, perivascular mononuclear cell infiltration, and glial cell proliferation, occurring mostly in the cord and the anterior horns. The sparseness of reaction in the cortex, with slight or absent meningeal involvement, is similar to human poliomyelitis.

Susceptibility to experimental infections.—Young mice are most susceptible to central nervous system infection. The intracerebral inoculation of mice under 30 days of age is likely to result in death without paralysis. Paralysis is most likely to develop in mice infected between 6 and 10 weeks of age (*6*).

Routes of infections.—All strains of the virus given intracerebrally will infect young mice, but only the more virulent strains such as Theiler's GDVII and FA strains readily infect them intranasally or intraperitoneally, though many more intracerebral, minimal infective doses are required for infection. It takes about 100,000 intracerebral, minimal infective doses to infect mice by the intranasal route, and about 10 million to infect them intraperitoneally (*4*). Virus administered by both the intracerebral and intranasal routes results in typical encephalitis and paralysis. Virus by the intraperitoneal route generally produces paralysis without encephalitis and with a lower mortality.

Olitsky (*3*) could not infect the central nervous system of mice by administering the virus intradermally, subcutaneously, through scarified skin, or by feeding. Nor does brain injury cause localization of the infection as in lymphocytic choriomeningitis where virus from other infected tissues tends to localize in injured brain tissue.

Diagnosis

1) Flaccid paralysis develops 7 to 37 days after intracerebral injection of the virus.

2) Characteristic microscopic lesions in the central nervous system, *i.e.*, necrosis of anterior neurons.

3) Positive intracerebral transfer to other mice with a 10^{-1} brain and cord suspension. (Brain and cord should be harvested in the first few days of paralysis. After that the virus may only be found in the cord (*2*).

4) Cross-immunity tests between the spontaneous central nervous system, Theiler's disease, and virus recovered from the digestive tract.

Method of recovering virus from the intestinal tract (*3*).—The intestinal contents of about 10 inches of small intestine from 3 to 5 young mice, 30 to 60 days old, are pooled. This material is ground with alundum, in physiological saline solution or broth in a 10 percent suspension, centrifuged for 15 minutes at 2500 r.p.m., filtered through a Seitz filter, and cultured. (A few bacteria usually do not interfere with the production of a typical infection.) Mice from 20 to 40 days of age are injected intracerebrally with .03 cc. of the suspension and are observed for 40 days.

Recovery of virus from the central nervous system.—In mice with central nervous system involvement and paralysis, the virus is found only in the brain and cord. It may be found in the cord as long as 1 year after infection and paralysis, but is less persistent in the brain (*6*). Consequently, brains harvested for virus passage should be obtained within the first few days of paralysis and passed intracerebrally to 30- to 60-day-old mice. In mice infected intracerebrally, but without paralysis, the virus has been recovered from the central nervous system up to 163 days later (*6*).

Immunity.—Some degree of immunity develops in survivors of intranasal or intraperitoneal infections, but they remain carriers for about 1 year. The serum of mice that have been paralyzed and that are immune to the intracerebral injection of virus will have acquired some neutralizing antibody (*6*). There is no serological relationship to human poliomyelitis (*6*). A natural immunity is acquired with age so that old mice are difficult to infect by any route.

Significance of the Disease

Most mouse colonies appear to be infected with Thieler's virus. With rare exceptions the virus is carried in the digestive tract and only about 1 mouse in 4,000 or 5,000 acquires a central nervous system infection. If the virus produces any recognizable pathologic change in the intestinal tract, the lesions have not been reported. Only young mice appear to be susceptible to invasion of the central nervous system, and those that become naturally infected either die or develop obvious and quite characteristic paralysis and show anterior horn lesions simulating those of human poliomyelitis.

Since most laboratory mice are used at the age when they are susceptible to central nervous system infection, care should be taken to observe each mouse individually while it is moving about to eliminate any that show evidence of paralysis or encephalitis. Mice should be

examined for muscular atrophy or weakness. Questionable mice should not be used.

The high incidence of the virus in all mouse colonies examined suggests the difficulties in obtaining breeders from which to start a clean colony and the need for strict isolation to maintain such a colony. These difficulties have caused most laboratories to be on the alert for individual mice with central nervous system symptoms, and to discard these, but otherwise to ignore the presence of the virus.

A careful study of the histopathology of the mouse digestive tract might well reveal changes due to the virus which should at least be recognized for what they are. Otherwise the reference to "normal control" becomes only a relative expression and leads to errors, especially in pathologic studies.

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Mouse Pox (Infectious Ectromelia)[1]

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Mouse pox is an infectious disease caused by a filtrable virus which is related to the other animal poxes. It was first described in 1930 by Marchal (*1*), at Hampsted, England, and has since been reported from France, Germany, Russia, Switzerland, and the United States (*2, 3*).

Mouse pox is highly infectious and often epizootic. All strains of mice are susceptible, though some are more so than others. Rats are resistant like rabbits and guinea pigs; however, it is possible to induce inapparent infection in these species which results in the formation of circulating antibodies. Several hundred thousand humans have been inadvertently inoculated with living mouse-pox virus in the course of murine typhus vaccination, but no untoward reaction has been reported (*2, 4*). Chick embryos may be readily infected by amniotic, suballantoic, or yolk-sac inoculation. Serial passage of the virus on the chick chorio-allantois makes it more infective for rabbits and guinea pigs but reduces its virulence for mice.

The virus particles are 100 to 150 mμ in diameter. The virus is destroyed at 55° C. for 30 minutes and, in the refrigerator, withstands 50 percent glycerol and 0.5 percent phenol for several months.

The virus has the same hemagglutination range as vaccinia virus, but it also agglutinates mouse erythrocytes (*5*). Serologic diagnosis employs serum from mice recovered from mouse pox, which should inhibit red-cell agglutination by vaccinia virus. To demonstrate the virus itself, portions of suspected liver and spleen are ground in small amounts of saline, and the mixture is inoculated intraperitoneally into susceptible mice. If virus is present, the mice will usually die in 4 to 6 days with gross lesions in the liver.

In its natural host, the mouse, mouse pox is a generalized disease, and the virus multiplies in cells of the skin, lymph nodes, liver, spleen, and often in other organs. Inclusion bodies develop in infected epidermal, liver, and spleen cells, and may also be present in the skin, heart, lung, intestine, pancreas, and salivary gland. The inclusions are eosinophilic

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and intracytoplasmic. The virus has no cell or organ specificity in either the mouse or the chick embryo, and, in tissue cultures, inclusion bodies have been found in organs and tissues arising from all three germ layers.

The acute disease is characterized by visceral lesions. Animals may die without apparent cause, appearing in good health until a few hours or a day before death. There may be no readily apparent external lesions, though inconspicuous primary lesions are sometimes present. In other instances, swollen eyelids, swollen pocked nares, and pocklike lesions on the body may be prominent signs. One death is usually followed by deaths of other contacts within a few days to a few weeks. The disease usually spreads throughout the colony in 6 to 8 weeks with a 50 to 95 percent mortality.

The first symptoms are nonspecific, consisting of ruffled hair and a puffy appearance of the face.

In a fulminating case the following gross autopsy findings may be present: *a*) pale, dirty-gray or putty-colored liver, or red liver mottled with white areas of focal necrosis; *b*) slightly enlarged spleen, sometimes with large white areas in longstanding cases; *c*) hemorrhagic appearance of the proximal inch or two of small intestine; *d*) excess fluid in the peritoneal cavity and fibrinous exudate covering the intestines; *e*) pulmonary congestion or consolidation, sometimes with excess pleural fluid. Microscopic anatomical findings may include necrosis of mesoblastic tissues and large eosinophilic intracytoplasmic inclusion bodies in epithelial cells of the skin and the intestine. The epidermal cells are swollen and vacuolated. Inclusion bodies are not always present in the fulminating disease. Hepatic necrosis is the dominant histologic lesion. It is always focal and random in distribution without any regular relationship to the liver architecture.

The chronic disease is characterized by necrosis of the extremities. Usually one foot becomes swollen and edematous, with serous fluid escaping from the surface and forming minute scabs. This progresses to gangrene and sloughing of the foot, leaving healthy tissue, or spreads to another foot, to the tail, or the mouth, perhaps eventually causing death. The chronic foot lesions must be distinguished from those caused by *Streptobacillus moniliformis* by cultural means. In recovered mice, focal necrosis of the spleen, and later focal scarring persist throughout life, and are a reliable index to previous infection. The liver lesions do not persist, but are replaced by regeneration of new liver cells without accompanying scarring.

The following statements on prevention and control of the disease appear in the official report of the Committee on Infectious Ectromelia of Mice (*6*):

"Prevention of the disease calls for strict isolation of the mouse colony from outside contamination. This also includes adequate facilities for handling the colony with a noncirculating system if air conditioning is practiced. It is possible that vaccination of individual mice by the scarification method or intranasal instillation using vaccinia virus or formalinized liver suspension from infected mice . . . may be of benefit. . . .

This is probably impractical in most cases except for particularly valuable inbred stocks.

"Control of the disease demands prompt recognition of infection. Infected colonies should be exterminated by destruction and incineration of infected animals and contacts. The quarters should then be sterilized.

"The committee recommends the following for the control of this disease:

a) Extermination of known infected colonies.

b) Destruction of materials harvested from infected colonies.

c) Isolation and self-imposed quarantine of colonies suspected of infection.

d) Prompt reporting of all suspected and proved infections.

e) Restriction of shipments of mice between investigators.

f) Treatment of all materials such as blood, serum, tissues, or ectoparasites harvested from mice, particularly from *foreign* sources, as potentially contaminated, and handling of such materials with all precautions necessary.

g) Familiarizing of scientists with the existence of legislation prohibiting the importation or interstate movement of ectromelia virus, or animals or materials potentially infected with it. [An Act approved February 2, 1903 (32 Stat. 792; 21 U. S. C. 111) and BAI Order 381 (9 Code of Federal Regulations, Parts 101–123), regarding the importation or interstate transportation of viruses or vectors (animals inoculated or exposed thereto) have been interpreted as applicable to infectious ectromelia. A permit from the U. S. Department of Agriculture is, therefore, required for the importation or interstate movement of ectromelia virus or materials infected with the virus.]"

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Pathology of Infection with the JHM Virus [1]

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I shall describe an interesting virus infection which suddenly appeared in our mouse colony, and which was studied rather thoroughly by Dr. Cheever, Mrs. Joan Daniels, Dr. Orville Bailey, and myself (*1, 2*). We called the new agent JHM virus, in honor of Dr. J. Howard Mueller, though in retrospect it seems a questionable compliment to have a malignant virus named after one.

The JHM virus is interesting because it produces a disseminated encephalomyelitis which is characterized by great destruction of myelin, and by other histopathologic features which I shall describe in detail.

Our studies began with finding two mice in the stock colony with flaccid paralysis of the hind limbs. They were 17 or 18 days old at the time, and belonged to the so-called Schwenkter strain of Swiss albino mice.

A 20 percent suspension of the brains of these mice was inoculated intracerebrally into four 22-day-old mice, one of which, after the 8th day, became ruffled and paralyzed in the hind legs. From this mouse consecutive passages were made, and the disease was maintained without further difficulty.

The disease in the experimentally infected animals was more or less as follows: The incubation period during early passages was 4 to 8 days, but after the 37th passage signs of illness became manifest after 24 hours, and death occurred usually within 36 hours. Titrations of late passage material gave an LD50 of $10^{-4.5}$ to $10^{-5.5}$.

In the earlier passages, paralytic symptoms prevailed, but as the virus gathered virulence, most of the animals died with predominantly encephalitic signs, *i.e.*, incoordination, weakness, and, occasionally, tremors and convulsions.

With other routes of inoculation than intracerebral, there was a delay in the incubation period and paralysis occurred more frequently. The disease could be readily induced by intravenous, subcutaneous, intranasal, and intraperitoneal inoculation, but only rarely by intramuscular injection. Virus was isolated from liver, spleen, lungs, and kidneys, as well as from nerve tissue, but not from intestinal contents or blood.

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[2] Deceased.

In addition to infecting mice, the JHM virus proved to be pathogenic for cotton rats, hamsters, and suckling rats but very slightly pathogenic for adult rats, and not at all for guinea pigs, rabbits, or monkeys. No serological relationship to Theiler's or other neurotropic viruses could be demonstrated (fig. 1).

The pathologic changes produced by this virus differ in several respects from those caused by other known neurotropic viruses. The most characteristic and important feature is that the JHM virus brings about extensive destruction of the white matter, tending to spare the ganglion cells and nerve fibers, both in brain and cord (figs. 2 and 3). Another distinctive feature is the frequent occurrence of necrosis of the liver (fig. 4), which has never been noted in infections with Theiler's virus or other neurotropic viruses. A third characteristic is the production of large multinucleated giant cells (fig. 5). These originate from a variety of cell types not only in the central nervous system, where they appear to derive from meningeal connective tissue cells, endothelium, fusion of histiocytes, and ependyma, but also in the liver, spleen, lymph nodes, and intestinal lymphatic tissue.

Since the principal interest in this new disease lies in the fact that the agent brings about a widespread and selective destruction of myelin both in brain and cord, I shall discuss demyelinating diseases, in general.

Demyelinization is a salient feature in the following diseases and conditions:

1) In the encephalitis which may follow various infectious diseases, notably measles, varicella, and variola, and small-pox vaccination or rabies immunization.

2) In acute disseminated encephalomyelitis of man and in multiple sclerosis.

3) In Schilder's disease.

4) In monkeys, as a spontaneous form of disseminated encephalomyelitis.

5) In so-called "allergic" encephalomyelitis, produced by injection of normal brain tissue or its components, usually with the aid of adjuvants.

In the postinfectious encephalitides, the loss of myelin is predominantly perivenular, whereas in the JHM disease no such perivascular localization was apparent. This is true also of the demyelinization in acute disseminated encephalomyelitis, and, of course, in multiple sclerosis. The perivascular localization is also a feature of the experimentally produced allergic disease and is accompanied by a cuffing of the vessels with inflammatory cells, which were not a conspicuous feature of the lesions in the JHM disease. Another differential point is that in our mice the myelin destruction was unaccompanied by the liberation of stainable fat in any quantity. King (*3*), in his study of dog distemper myelitis, has called attention to this form of myelin degradation, and Morrison (*4*) has shown

that the phosphorylcholine moiety of lecithin may be split off by *Clostridium welchii* lecithinase without the liberation of stainable fat. We have noted in our sections a striking hydropic swelling of the oligodendroglia nuclei, and since these cells are regarded as the functional equivalent of the Schwann cells in their trophic influence upon the integrity of the myelin sheaths, we have toyed with the idea that the virus may localize selectively in these elements of the central nervous system, and that their injury may bring about a secondary disintegration of the myelin. Bailey, who has continued to pursue cytologic studies of the JHM virus, writes that he has reached the same conclusion, *viz.*, the primary effect is upon oligodendroglia. But direct proof of such an effect is lacking.

One thing is reasonably certain: The myelin destruction in this disease is a direct result of the action of the infecting agent and is not brought about indirectly by the material used for inoculation. Although "allergic" encephalopathy has been experimentally produced in mice by Olitzky and Yager (*5*) by intramuscular injection of normal brain-tissue suspensions fortified by addition of killed tubercle bacilli and mineral oil, we found it impossible to do this by injection of normal brain tissue alone. Furthermore, the lesions could not have been caused by something derived from normal brain tissue because they followed also upon injections of infected liver and other organ suspensions. The time relations also speak against a possible "allergic" etiology, since the demyelinating lesions began soon after injection and reached their peak after 7 days. There was thus no lag period for the production of antigens, whereas in the experimental "allergic" disease, periods of 14 to 47 days have elapsed before the appearance of encephalitic symptoms. Again, many brain suspensions containing other neurotropic viruses may be injected without producing the marked myelin destruction characteristic of this disease.

Just how this extensive myelin destruction is brought about, still awaits explanation. Does the virus carry with it enzymes which destroy myelin, or does it destroy the sustentacular elements necessary for its preservation? Does it activate some pre-existing enzyme system or block an inhibitor? These are problems for the future. At best, the finding of a viral agent having such a striking effect upon the white matter should be useful in further work along this line.

Interesting as this affinity of the JHM virus for the white matter is, it is by no means solely distinctive of this particular agent. For example, the myocarditis EMC virus isolated by Helwig and Schmidt (*6*) from a gibbon and chimpanzee, in 1944 in Florida, when adapted to mice, produces extensive demyelinating lesions in the cord. Also, the agent recovered from cases of acute disseminated sclerosis in man, by Margulis, Soloviev, and Schubladze (*7*), caused demyelinating lesions in the spinal cord of mice. The lesions of brain and cord in the nervous form of canine distemper are said to be accompanied by severe myelin destruction like those in the still debatable "foot-pad disease" of MacIntyre, Trevan, and Montgomery (*8*), and of Inness (*9*). Scherer (*10*) has also described an acute disseminated sclerosis in dogs in which the nerve ele-

ments are faultlessly preserved, but in which there are extensive patches of myelin destruction in the white matter.

Since the completion of our work on JHM virus, Morris and Aulisio (*11*, *12*) of the Armed Forces Graduate Medical School have published a brief abstract in the *Federation Proceedings* and a short paper in a "Symposium on Hepatitis Virus," under the auspices of the National Association of Sciences. They have recovered a similar murine agent (H747) during work in Japan, which they regard as representative of a new group which they call *hepato-encephalitis viruses*. Antisera against their strain neutralized not only the homologous virus but JHM and the mouse hepatitis virus of Gledhill and Andrewes (*13*, *14*) and several other strains. In a recent letter from Dr. Niven, she states that they have isolated several new strains of hepatitis-producing virus that appear to be antigenically related to JHM, and which elicit similar histologic changes. She does not mention whether demyelination is a feature of the central nervous system lesions.

During the past summer, paralysis was observed in members of several litters in a single cage, and, from these, Mrs. Daniels has again isolated an agent that produces demyelinating lesions, liver necrosis, and giant cells. We have not as yet established the identity of this strain with JHM but, judging from the pathology, it should be closely related.

Pathology of Infection with DA Virus

In collaboration with Mrs. Joan Daniels and Sheila Richardson, I have had the opportunity to study the lesions provoked by another strain of virus (DA), originally isolated from the brain of mice that had shown spontaneous paralysis over a period of 2 months (*15*). This strain had limited virulence for mice; the MPD/50 (50% mean paralyzing dose) even after repeated passages did not exceed $10^{-3.2}$ in 3-week-old mice, or $10^{-4.7}$ in sucklings. Older animals were even less susceptible, but some of them did develop paralysis. Histologically, the brain showed patchy meningitis, lesions of the blood vessels in the form of proliferations of microglia cells or histiocytes about the capillaries, and small typhuslike nodules not apparently related to the blood vessels. Only in young sucklings did one find more extensive areas of necrosis.

Very interesting lesions were found in the cord of older mice that had survived the initial injection and had become more or less chronically paralyzed. In these, the lesions were restricted to the white matter, and again consisted of patchy areas of myelin destruction with formation of microcysts, together with proliferation of microglia, hypertrophy of astrocytes, and apparently some increase or condensation of neuroglia fibers (fig. 6). The lesions of the gray matter were trivial—some perivascular cuffing or slight accumulation of microglia. The neuraxons, as was the case with the JHM lesions, were distorted but not destroyed. Unlike the JHM disease, there were no liver lesions and no giant cells. Also the ventral nerve roots were frequently degenerated, and this could

not be accounted for by destruction of motor ganglion cells (fig. 7). And still another interesting feature was the occurrence of myositis in trunk and back muscles with lesions indistinguishable from those caused by Type I Coxsackie strains.

Again the question arose as to whether the demyelinating lesions were in the nature of a sensitization phenomenon, developing in the course of the protracted disease, or were due to a direct action of the virus. In favor of the latter view was the fact that we were able to recover virus, even after 4 months' postinoculation.

The relation between this agent and certain other known strains of Theiler's mouse encephalomyelitis, particularly the low virulent strain 4727, has not been determined with finality. So far as we are aware, demyelinating lesions have never been described as occurring in spontaneous or experimental infection with Theiler's virus, but we have seen these lesions in one mouse inoculated with strain 4727 at the age of 5 weeks and sacrificed after having been paralyzed in the hind limbs for a month. So it would seem, from this single observation, that some Theiler strains of low virulence are capable of inducing chronic paralysis accompanied by demyelinating lesions of the white matter.

Demyelinating Disease in Rats

I shall describe briefly another demyelinating disease which we have found in two adult rats. When these animals were about 9 weeks old, it was noted that they were dragging their hind limbs, and in the course of the next few weeks the paresis became progressively more severe but it never became complete. Although locomotion was accomplished by dragging the body with the forelegs, when the hind limbs were stimulated, they were still able to perform irregular movements. There were no tremors, encephalitic signs, and their general condition, despite the paresis, remained good. The rats were killed at the age of 84 and 105 days (*16*).

Sections of the spinal cord disclosed a most extensive demyelinization of ventral and lateral tracts at all levels, with formation of microcysts (fig. 8). There was complete absence of inflammatory reaction. The ganglion cells were unaffected, and no changes were seen in dorsal or ventral nerve roots. All parts of the brain were free from encephalitic or demyelinating lesions, and no significant changes were found in other viscera.

We were unsuccessful in attempting to pass a viral agent by injection of fresh cord suspension into suckling or older rats. It is possible that, because of the long duration of the illness, the original inciting agent was no longer present. Be that as it may, the cause of this interesting disease was not discovered, and no subsequent cases appeared in our colony. It could be shown that the serum of the adult rats contained no neutralizing bodies against the JHM virus, and, because there were also many histopathologic differences, we may conclude that this is an unrelated disease. But it offers another good example of the occurrence of demyelinating lesions in animals.

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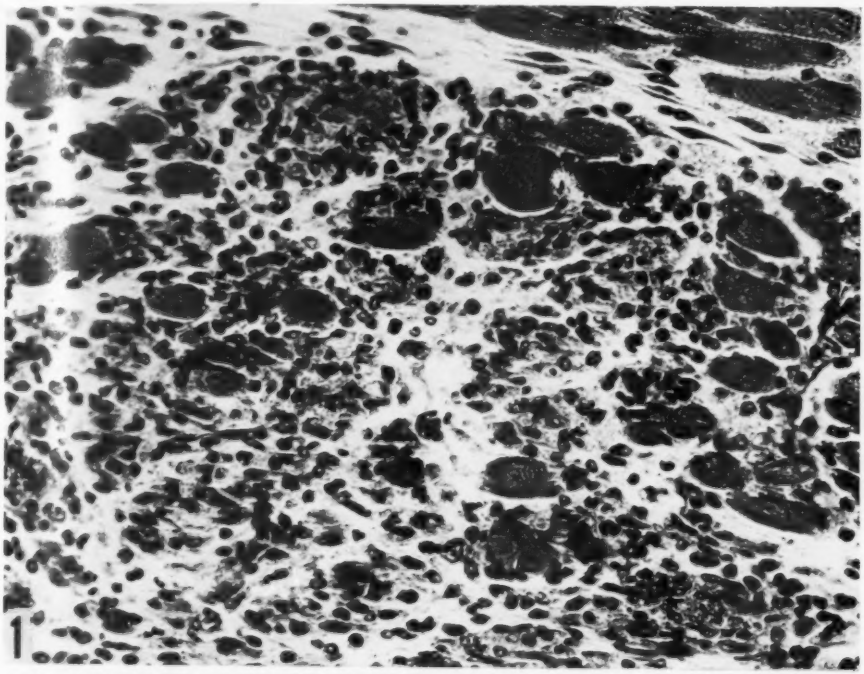
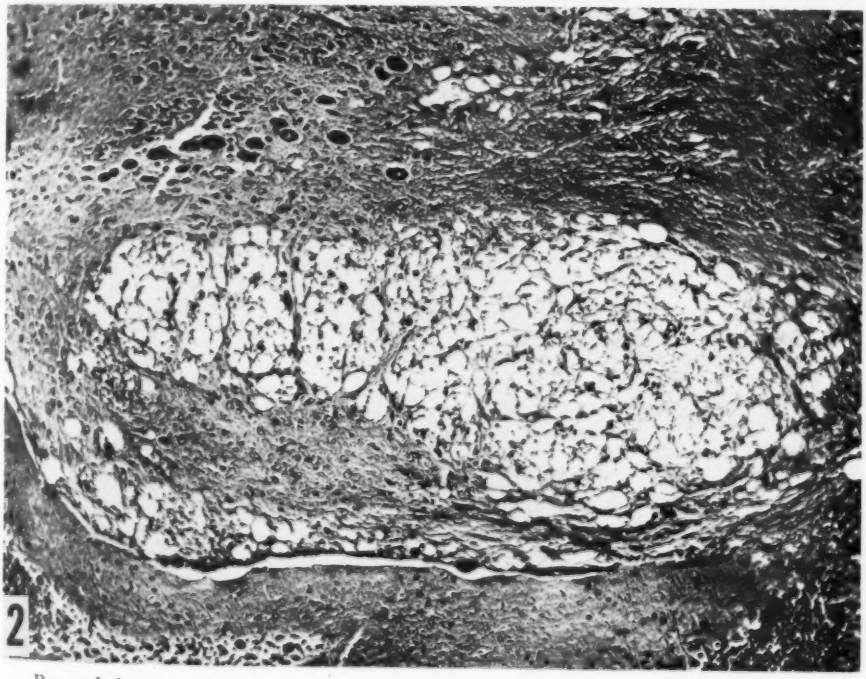
Plate 74

Figure 1.—Myositis in suckling mouse produced by FA strain of Theiler's virus. Killed 6 days after intracerebral injection of 10^{-2} infusion of infected mouse brain.

Figure 2.—JHM virus. Microcystic degeneration in white matter of cerebellum, 11 days after intracerebral injection.





Pappenheimer

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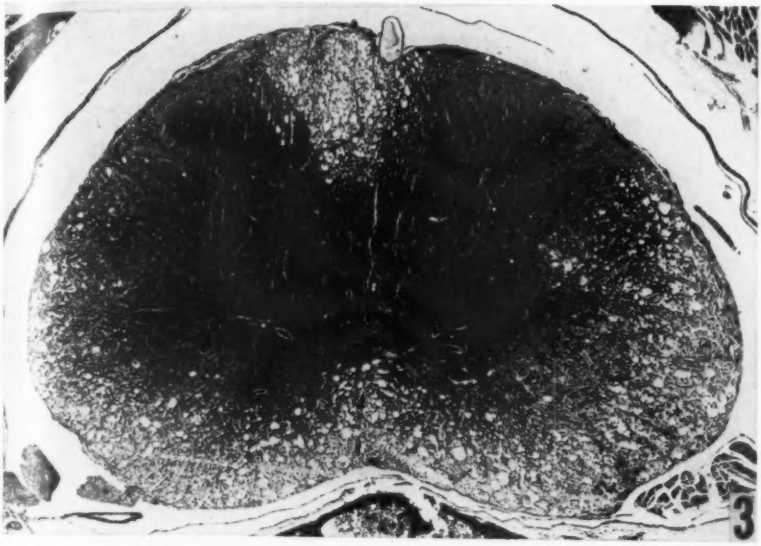
PLATE 75

FIGURE 3.—JHM virus. Extension demyelination in spinal cord, 1 month after intracerebral inoculation.

FIGURE 4.—JHM virus, 49th passage. Necrosis of liver, 4 days after intravenous injection. (*From* Bailey *et al.*, J. Exper. Med. 90: 195–212, 1949, plate 16.)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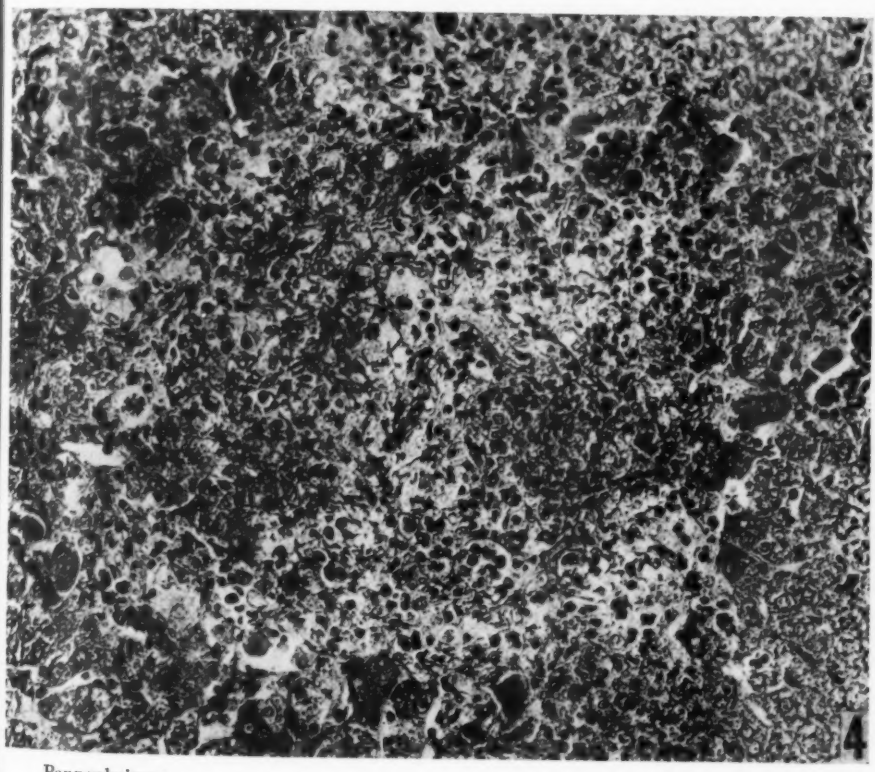
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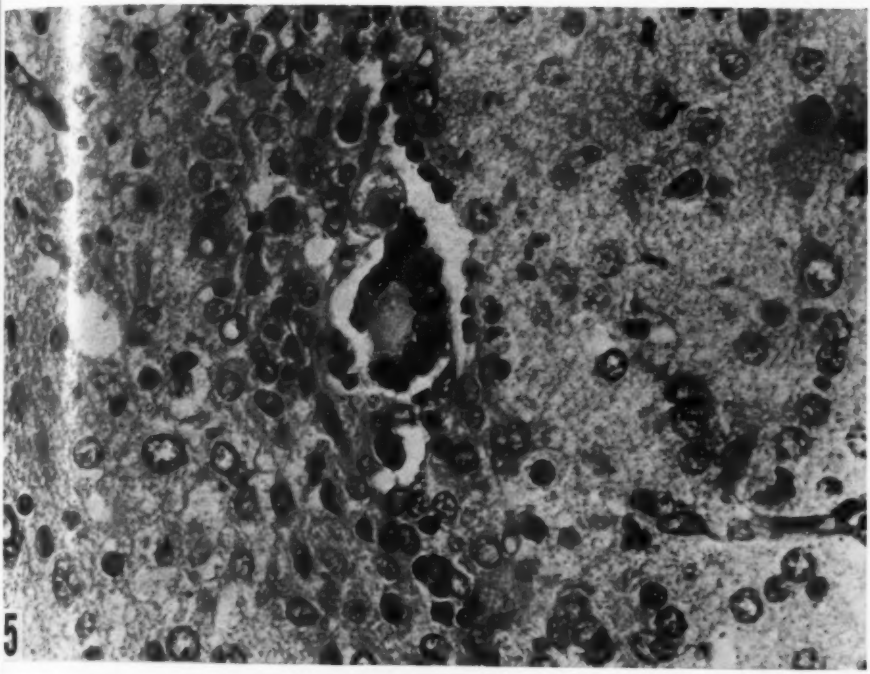
PLATE 76

FIGURE 5.—JHM virus. Large giant cell of ependymal origin in an area of periependymal necrosis, 4 days after intracerebral injection.

FIGURE 6.—DA virus. Weanling mouse inoculated intracerebrally with 10^{-2} infusion of infected brain. Sacrificed after 71 days. Myelin destruction in lateral tracts of spinal cord. (*From* Daniels *et al.*, J. Exper. Med. 96: 517, 1952, plate 23.)



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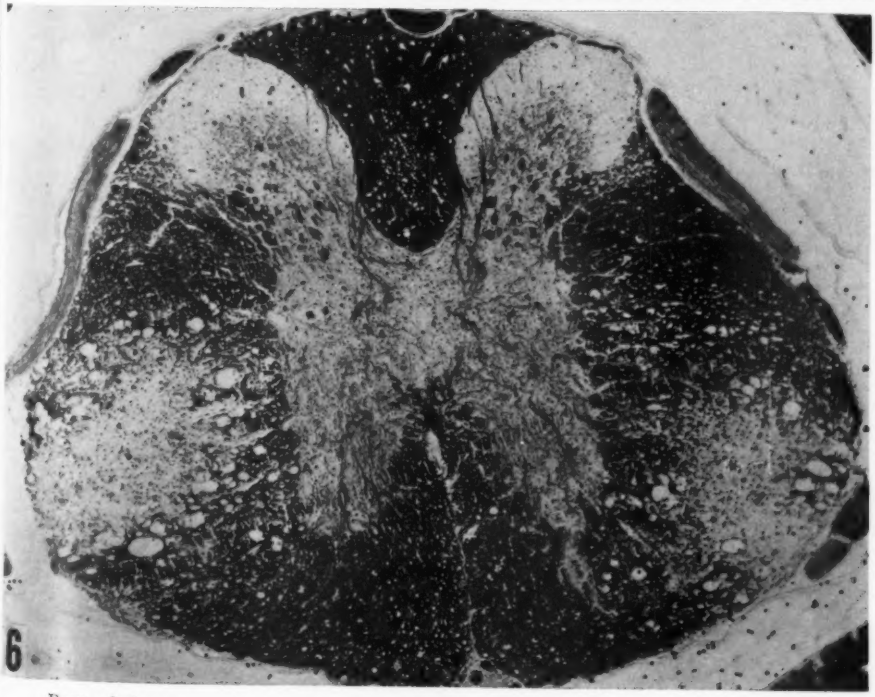

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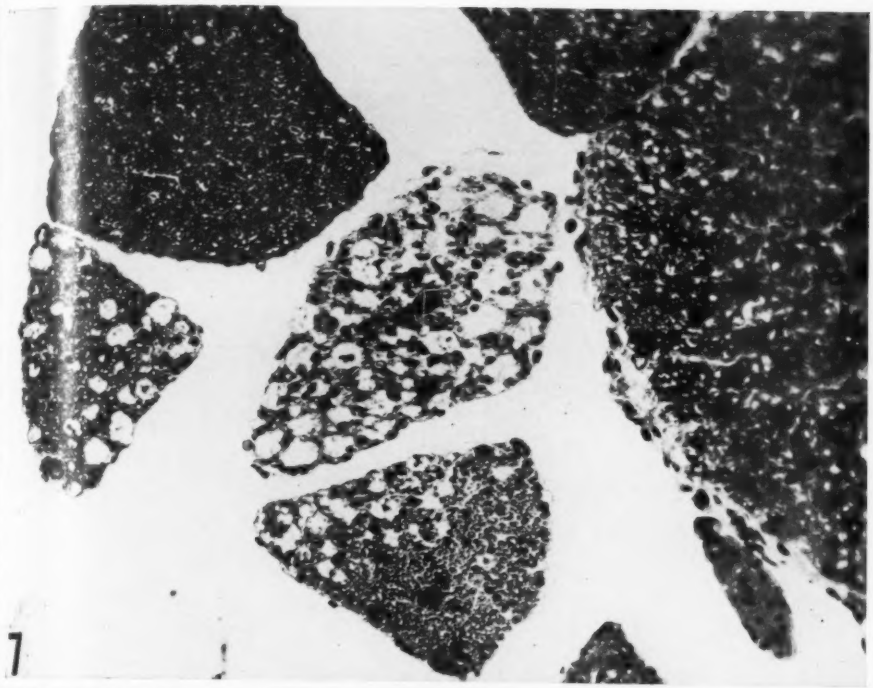
PLATE 77

FIGURE 7.—DA virus. Mouse inoculated intracerebrally with infected amniotic fluid. Killed 33 days later. Degeneration of anterior neuron roots in spinal cord. (*From* Daniels *et al.*, J. Exper. Med. 96: 517, 1952, plate 22.)

FIGURE 8.—Spontaneously paralyzed rats. Extensive demyelination in white tracts of spinal cord. (*From* Am. J. Path. 28: 351, 1952.)



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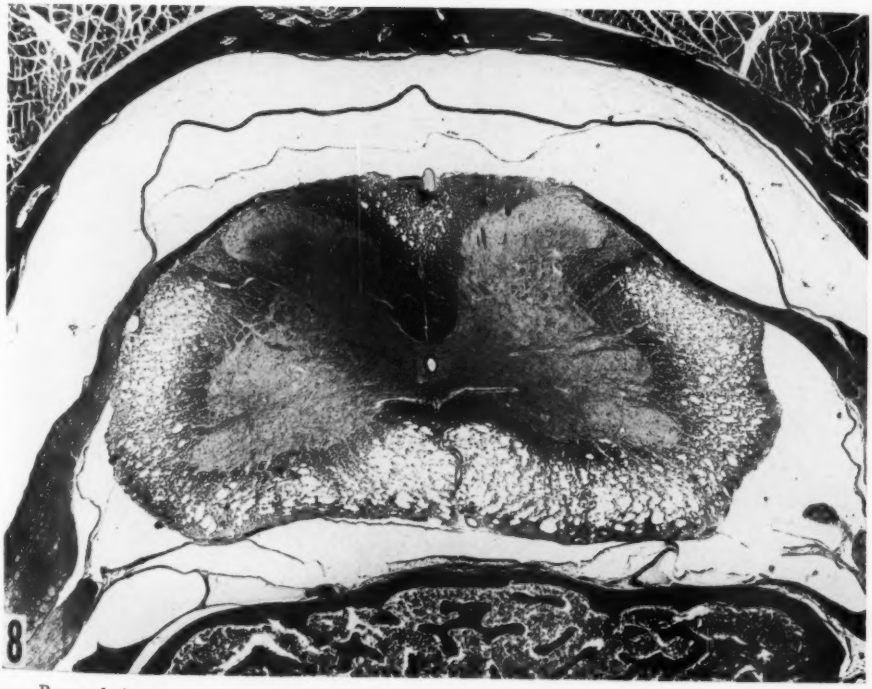

8

Tyzzer's Disease [1]

LEON Z. SAUNDERS,[2] *DVM, Brookhaven National Laboratory, Upton, Long Island, New York*

An infectious disease associated with the presence of a rodlike organism, *Bacillus piliformis*, and characterized by focal necrosis of the liver, was first described by Tyzzer (*1*) in 1917 in a colony of Japanese waltzing-mice at Harvard Medical School. Since then, outbreaks in laboratory mice have been observed in China (*2*), England (*3, 4*), and Sweden (*5*). Dr. W. J. Hadlow (*6*) has observed sporadic cases in Montana. Mice are the only animals in which natural infection occurs. Encephalitis can be produced experimentally in rats, rabbits, and hamsters by injecting the organism (*6*), but the liver disease cannot be reproduced in these species.

B. piliformis appears to live as a saprophyte in many mouse colonies. It is not until the mice are subjected to some experimental procedure or to adverse environmental conditions that clinical symptoms arise. Hence the presence of the disease may be unsuspected until an experiment is started, and then symptoms begin to appear in the experimental animals. In Tyzzer's (*1*) experience the adverse factor was the formation of tumors. In Gorer's (*3*) report the stress factor was again the injection of a carcinogenic agent. Gorer found that the disease occurred only in mice in which tumors developed. In Gard's (*5*) experience, the adverse conditions were a combination of the heat of the summer months and a mushy diet of bread, wheat-germ flour, and fish-liver oil soaked in whole milk. He was able to control the outbreak when he replaced the wet food with dry pellets. This preventive measure had originally been recommended by Tyzzer, who found that dried food was much less easily contaminated with feces by the mice. Rights, Jackson, and Smadel (*4*) found that the adverse environmental factor in their outbreak was crowding. Outbreaks occurred in two successive years when the breeding stock was subjected to overcrowding, and the disease returned to a sporadic status when the animals were provided with adequate quarters. Similarly, Hadlow (*6*) has noted that cases of Tyzzer's disease will occur if mice are kept in a cage for a long period without changing the bedding. These

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Journal of the National Cancer Institute, Vol. 20, No. 5, May 1958
459041—58——5

sporadic cases can be controlled by the simple expedient of providing the mice with freshly bedded cages at suitable intervals.

The disease may occur in either enzootic or epizootic form. It is, of course, the latter that wreaks havoc in a mouse colony. Tyzzer lost the whole of his Japanese waltzing-mouse colony, a total of 79 animals, over a period of 5 months. Gard's epizootic involved more losses by death in a shorter time, since 1,200 young mice died in less than 4 weeks. Gard did not state the total number of mice in his colony or the number that became ill, so there are no exact morbidity or mortality data.

Tyzzer (*1*) found that common laboratory mice did not die during the epizootic in his Japanese waltzing-mice. He postulated that the infection had been carried in latent form by the common mice that had introduced it among the Japanese mice; also that only Japanese mice were susceptible. The genetic susceptibility and resistance of mice to this disease was later studied by Gowen (*2*) who found that the susceptibility of Japanese mice was not linked to the waltzing gene, coat color, or to sex. Gowen obtained 80 percent takes on attempted experimental infection in Japanese waltzing-mice and rather lower percentages in other mice. Observations by all subsequent workers have shown that the ordinary laboratory mouse, *Mus musculus*, is just as susceptible under the proper conditions as the Asian mouse, *Mus bactrianus*.

B. piliformis is a slender, delicately curved rod with tapered ends, which is found within living liver cells. These rods are from 10 to 40 μ long and 0.5 μ or less in thickness. They are gram-negative and appear banded when stained with fuchsin. They may also be demonstrated in Giemsa-stained sections or impression smears of liver. Sometimes the rods may be found in the intestinal epithelium as well as in liver cells. Tyzzer reported that he found them in the gallbladder and in the bile. This organism is a rather interesting one inasmuch as it seems to live only intracellularly. Tyzzer's observation that it forms spores in the cells has not been confirmed by subsequent investigators. No one has succeeded in growing *B. piliformis* on artificial media. Rights and associates were successful in cultivating it on tissue cultures. They used serum-agar slopes with tissue from minced chick embryos. Although organisms were present in their primary cultures, which were virulent for mice, subcultures contained only a few organisms and these were completely avirulent. The use of embryonic mouse tissue in the agar slopes was more successful. This resulted in an abundant growth of organisms and the first subculture was virulent for mice. However, second, third, and subsequent subcultures, although rich in organisms, failed to produce infection in mice.

The infectivity of mouse liver tissue, either in saline suspension or as the organ itself, is destroyed by storage in the frozen state for longer than 2 weeks. At $-20°$ C. or $-70°$ C. for a few days, the agent can still be recovered.

The part that the *piliformis* organism plays in the etiology of mouse liver disease is not clearly established, though the investigators have found

the organism to be invariably present. It is not known whether it is the sole agent of the disease or whether an associated agent is responsible and the *piliformis* organism is merely a saprophyte that accompanied it. It may be that *B. piliformis* is present in many normal mouse livers, and when the liver is damaged by some other agent, the bacteria are able to proliferate to numbers that are readily seen microscopically. It is evident that there is need for further research on the etiology of Tyzzer's disease.

Recovered animals appear to have immunity to subsequent infection, either natural or experimental. There is also evidence that they confer a passive immunity to their young. In the outbreak described by Gard, the sucklings usually remained well, while the weanlings at an age of approximately 3 weeks exhibited symptoms.

The symptoms are those of diarrhea of 2 to 3 days' duration, followed by anorexia and weight loss. Death occurs after 2 or 3 days more, and among young animals there may be few survivors. In older mice the course of the disease is more protracted and the mortality rate is low. The appetite may persist even while diarrhea is present, but growth is retarded. Adult mice may sometimes be found dead without having shown any symptoms. If the fur is removed from the abdomen, the liver lesions can be observed through the abdominal wall.

The gross lesions are circular grayish-white foci 1 to 2 mm. in diameter on the surface and cut surfaces of the liver (fig. 1). Histologically each focus consists of a central necrotic mass surrounded by a peripheral zone of neutrophils and lymphocytes (fig. 2). The surrounding liver cells show fatty metamorphosis. Numerous bacilli are present around the necrotic foci (fig. 3) but are seen in intact liver cells as well. A branch of the portal vein in the center of each focus indicates their embolic origin from the intestine. The mesenteric lymph nodes are edematous and congested and occasionally contain small abscesses. No lesions occur elsewhere, though the *piliformis* organism can be found in intact intestinal epithelium in sections. Intracerebral inoculation of affected liver causes encephalitis in the species mentioned above, but this lesion does not occur in natural infection.

Prevention and control depend on preventing or removing the conditions that are discussed above as predisposing factors to the disease. It would appear that in their absence the disease agent, while present in many mouse colonies, is not sufficient in itself to cause the disease.

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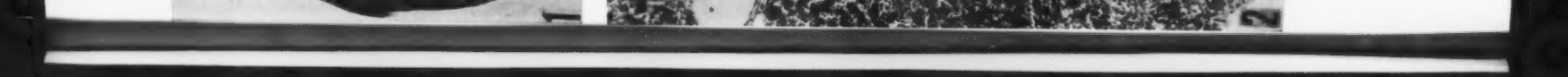
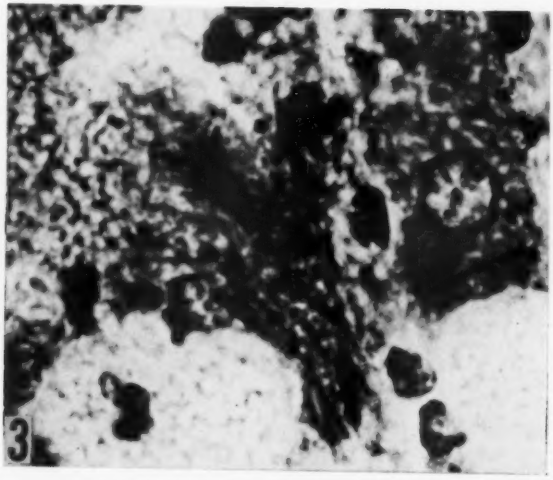
PLATE 78

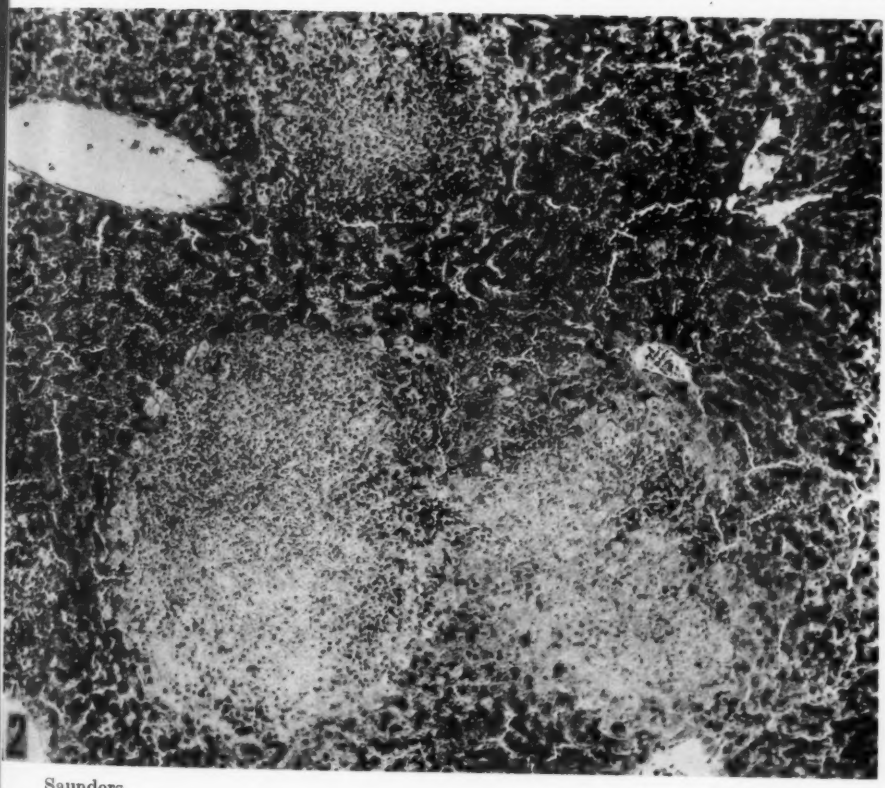
FIGURE 1.—Liver of a mouse affected with Tyzzer's disease showing necrotic areas. (*From* Tyzzer, J. Med. Res. 38: 307, 1917.)

FIGURE 2.—Section of mouse liver showing necrotic foci with peripheral leukocytic infiltration. (Tissue—courtesy of Dr. W. J. Hadlow, Hamilton, Montana.)

FIGURE 3.—Section of mouse liver infected with *Bacillus piliformis* organism. (Tissue—courtesy of Dr. W. J. Hadlow, Hamilton, Montana.)







459041—58——6

Myositis in Guinea Pigs [1]

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Myositis in guinea pigs was observed at Brookhaven National Laboratory in the spring of 1955. It appeared in a group of 50 newly purchased animals about a week after their arrival; the morbidity was about 75 percent.

The disease is characterized clinically by swelling and pain in the large muscle groups of the hind legs. The hind legs are kept flexed and the guinea pigs drag themselves around by their front legs. The appetite is maintained for 3 to 4 days, after which it wanes; about this time the front legs become involved. Within 3 or 4 more days, the animals become prostrated and die, apparently of inanition.

Gross lesions.—Animals killed in the early stages of the disease had ecchymotic hemorrhages in the large muscle groups of the hind legs, including the medial as well as the lateral muscles. The tissue surrounding the hemorrhagic areas was edematous, the edema involving the subcutis as well as the muscles. The lymph nodes draining affected areas were enlarged and also edematous. In the later stages there were similar lesions in the musculature of the forelegs, abdomen, and thorax.

Histologic lesions.—Hemorrhage and edema of the skeletal muscles are the most prominent changes; in addition, there is infiltration of the muscles with neutrophils, eosinophils, lymphocytes, and macrophages (fig. 1). Zenker's degeneration occurs but is a minor feature, since most of the muscle fibers are well preserved even when surrounded by inflammatory cells. In some areas of muscle there is proliferation of fibroblasts, while in others, there are phagocytes containing blood pigment. Peripheral nerves appear to be unaffected even when seen in areas of severe myositis. Histologic changes are not recognized in the brain, spinal cord, salivary glands, bones, pancreas, liver, or kidneys. Focal lymphocytic infiltration was present in the adrenal medulla of three animals. Inclusion bodies were not seen.

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[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1955.

[2] I am indebted to Dr. E. Dougherty III, Director of the Cornell Duck Disease Research Laboratory, for undertaking the cultural and animal inoculation work. The work conducted at Brookhaven National Laboratory was under the auspices of the U. S. Atomic Energy Commission.

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Etiology.—No pathogenic bacteria could be cultured from spontaneous cases, and no organisms could be demonstrated by staining muscle sections for bacteria, protozoa, and fungi. Frozen muscle from affected animals was transferred to another laboratory and inoculated into guinea pigs, intraperitoneally and intramuscularly. The disease appeared in the experimental animals 5 to 7 days following inoculation by either route. It could not be produced with muscle from spontaneous cases in a second attempt, nor could the artificial disease be passed on to additional animals. Attempts to pass the disease to embryonated hen eggs by inoculation of frozen muscle mixed with antibiotics appeared to be successful in killing embryos in the first passage; but the agent, if any were present, could not be passed further. Thus, attempts to determine the cause, while suggestive of infection at first, ended equivocally.

Differential diagnosis.—While the animals appear to be paralyzed, it is probably pain caused by the inflammation that makes them reluctant to use their legs. In the later stages, contraction caused by the fibroblastic proliferation may contribute to the flexion of the limbs. On a clinical basis, the prominent swellings on the legs serve to distinguish myositis from vitamin E deficiency and scurvy, and from diseases in which true paralysis occurs. Among the latter, nonsuppurative meningoencephalomyelitis is present in the spontaneous virus infections described by Römer (*1*), and by deGasperi and Sangiorgi (*2*). Nonsuppurative meningitis is present in experimental infection by lymphocytic choriomeningitis (*3*) and by salivary-gland virus (*4*). In the epizootic paralysis described by Holz (*5*), and in the case seen by Frauchiger (*6*), purulent meningitis was the underlying lesion. Myositis can thus be distinguished histologically from the above paralytic diseases by the absence of lesions in the central nervous system. This lack of lesions, together with the absence of the parasites, distinguished it from paralysis due to toxoplasmosis (*7*). Experimental infection with Pellissier's virus (*8*), which results in convulsions rather than paralysis, is also characterized by meningoencephalitis, and thus differs from myositis. In some respects the lesions of guinea-pig myositis are like those of Coxsackie infection in mice (*9*); however, none of the agents of the Coxsackie group are known to infect guinea pigs.

In experimental muscular dystrophy of guinea pigs due to vitamin E and other deficiencies, the lesions are primarily degenerative rather than inflammatory (*10*). Grossly, there are white focal lesions in the muscles resembling fish flesh, and the salient microscopic lesion is Zenker's degeneration. While intercostal hemorrhages have been described in scurvy of the guinea pig (*11*), the lesions of guinea-pig myositis do not occur in scurvy. Other scorbutic lesions, such as bone changes and grossly visible hemorrhage in the cecum and intestine, are not seen in myositis. The natural disease occurred in animals on a balanced laboratory diet supplemented daily with fresh lettuce; the experimental cases were produced in animals similarly well nourished.

Summary

A disease characterized by inflammation of the skeletal muscles occurred in a colony of guinea pigs. The cause was not determined, but it could be distinguished histologically from other diseases of guinea pigs which are characterized by impaired locomotion.

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PLATE 79

FIGURE 1.—Quadriceps femoris muscle of guinea pig showing infiltration by inflammatory cells. Hematoxylin and eosin.

Saunders 903

Salivary-Gland Virus Disease of Guinea Pigs [1]

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Salivary-gland virus disease of guinea pigs appears to be host-specific, and most domestic guinea pigs are believed to be susceptible. Clinical symptoms and gross lesions have not been observed in natural cases of the disease. The infection can be detected only by microscopic examination of salivary-gland tissues. Intranuclear and, more rarely, cytoplasmic inclusion bodies within the ductal epithelium are regarded as diagnostic.

Unusual cytoplasmic and intranuclear bodies within the ductal epithelium of salivary glands of guinea pigs were described by Jackson (*1*) in 1920. She considered the bodies to be intracellular stages of a protozoan parasite, most likely a coccidium. In 1926, Cole and Kuttner (*2*) reported the disease, identifying the etiologic agent as a filtrable virus. They transmitted the disease to noninfected guinea pigs by the injection of filtrates of macerated salivary glands from infected animals.

This virus disease of guinea pigs has been recognized in England and the United States and is assumed to be widespread in both countries. In published surveys it has been estimated that 70 to 80 percent of guinea pigs 6 months of age and older are infected.

The exact mode of spread of the infection is not known, but the observation of intracellular bodies within the ductal epithelium of the salivary glands and more rarely in the epithelium of the renal tubules suggests these organs may function in its transmission.

The many routes by which susceptible animals may be inoculated with the virus suspension include the subcutaneous, intraperitoneal, intraglandular, intracerebral, intramuscular, and intratracheal.

Susceptible animals inoculated intracerebrally show a rise in temperature of 3 or 4° F. within 48 hours. The temperature remains at this level throughout most of the course of infection but falls rapidly to subnormal levels preceding death (*2*). Within 72 hours after inoculation, anorexia and other manifestations of the disease occur in various combinations. Ptyalism is common, and the hair coat becomes dull and raised. At this stage the animals move with reluctance. Within 96

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hours of inoculation, tremors and slight convulsive movements are common. As the disease progresses the animals grow progessively weaker; their movements are jerky and lack coordination. Flaccid paralysis of the hind limbs with inhibition of the anal and urinary constrictor muscles is frequently noted. Many animals are moribund or dead by the 6th day.

Most reports indicate that clinical symptoms develop only after intracerebral inoculations, but Rosenbusch and Lucas (*3*) isolated a salivary-gland virus that caused death when inoculated by any route. However, nervous symptoms were limited to guinea pigs inoculated intracerebrally. This virus killed susceptible animals within 6 to 26 days, regardless of the mode or site of inoculation.

Pathologic Observations

Gross pathologic changes, usually limited to animals inoculated intracerebrally, are primarily congestion and inflammation of the meninges at the site of inoculation. Rosenbusch and Lucas (*3*) observed congestion, hemorrhage, and necrosis at all sites of inoculation.

Fetuses *in utero* were inoculated with virus suspensions both intracranially and intraperitoneally during the 4th and 5th weeks of gestation and delivered by cesarean section 21 days later (*4*). Upon gross examination petechiae were seen throughout the subcutis and most of the viscera. Small foci of necrosis were often observed in the liver. Occasionally the brain and meninges were hyperemic, with scattered hemorrhagic foci.

Microscopic changes limited to the salivary glands and the kidneys are observed in the tissues of animals having the natural disease. Affected salivary glands contain intracellular inclusion bodies in the ductal epithelium, and frequently the adjacent glandular parenchyma is infiltrated by lymphocytes and large mononuclear cells (figs. 1 and 2). Changes commonly occur in tubules and parenchyma at the periphery of involved glands and appear to have a special affinity for the serous portions of the submaxillary glands (*5*). In tubules in affected areas one or several of the lining cells in any one cross section may be enlarged, some to as much as 4 times their usual size. These altered cells may occupy their usual location or may appear to be partially or completely detached and extruded into the lumen. In hematoxylin and eosin preparations the cytoplasm of the enlarged cells is granular and mildly basophilic, resembling that of their unaltered sister cells. Inclusion bodies are sometimes seen in the cytoplasm of affected cells but intranuclear inclusion bodies are much more frequent. They vary in size, are acidophilic to basophilic, and usually are surrounded by a halo, with the nuclear chromatin marginated against the nuclear membrane. When present, cytoplasmic inclusions are best demonstrated in Giemsa preparations where they appear as spherical masses of scattered or clumped basophilic granules at the luminal side of the cell. The epithelial cells of the proximal and distal convoluted tubules of the kidneys may contain intranuclear and cytoplasmic inclusion bodies closely resembling those of the salivary glands.

The affected cells of the convoluted tubules, however, differ in that they do not appear to become greatly enlarged (*5*).

In inoculated susceptible animals the microscopic changes are similar to those observed in natural infections except that the site of inoculation becomes infiltrated by lymphocytes and large mononuclear cells. The nuclei of many of the large mononuclear cells contain inclusion bodies like those observed in the salivary glands and kidneys.

In animals inoculated intracerebrally, the lesion at the site of inoculation is primarily a local inflammation of the meninges in which the infiltrating cells are like those at other sites of inoculation. Similar changes usually are seen throughout the entire length of the spinal cord. Encephalomyelitis is more rarely encountered (*1*).

In fetuses inoculated *in utero*, the typical infiltrating cells and their intranuclear inclusion bodies are widely disseminated, particularly in the meninges, liver, and placenta (*4*).

When susceptible animals are inoculated subcutaneously, typically enlarged cells containing intranuclear inclusion bodies are demonstrable in the salivary-gland ducts as early as 13 days following inoculation (*3*, *6*).

Immunologic Features

Suspensions of the virus maintained at temperatures slightly above freezing for periods up to 11 days produced characteristic signs, symptoms, and lesions when injected into susceptible animals. Suspensions of the virus have been rendered noninfective when subjected to a temperature of 54° C. for 1 hour (*2*).

Seven successful consecutive passages of the virus have been completed in guinea pigs. In each instance a suspension of the salivary glands of the infected animal was used to inoculate the next animal in the series. A slight decrease in virulence was observed after each passage (*6*).

Colostrum does not produce recognizable protection for newborn animals against the disease. Immune serum, however, may prolong the survival period of animals inoculated intracerebrally. One report indicates an increase of 3 to 6 days in survival time of guinea pigs given immune serum and virus concurrently as compared with those given the virus alone (*7*).

Although the virus grows in tissue cultures, no successful subcultures have been reported.

Attempts to transmit the disease to rabbits, rats, cats, chickens, pigeons, dogs, mice, hamsters, and *Macacas* rhesus monkeys have been unsuccessful (*6*, *8*).

Although the disease is not likely to constitute a threat to animal colonies, it is reasonable to assume that such a virus could pass the natural defense barriers on rare occasions, and persons maintaining guinea-pig colonies should be alert to this possibility.

Since the exact methods by which the virus is spread are not known, instructions for maintaining a colony free of the disease are necessarily

incomplete. Several workers believe that the incidence of colony infection is directly related to the degree of sanitation practiced.

Summary

Salivary-gland virus disease of guinea pigs is apparently mild and specific for its natural host. Its presence is readily detected by histopathologic examination of tissue from diseased salivary glands in which the characteristic hypertrophy and intranuclear inclusion bodies are observed in the cells of the ductal epithelium.

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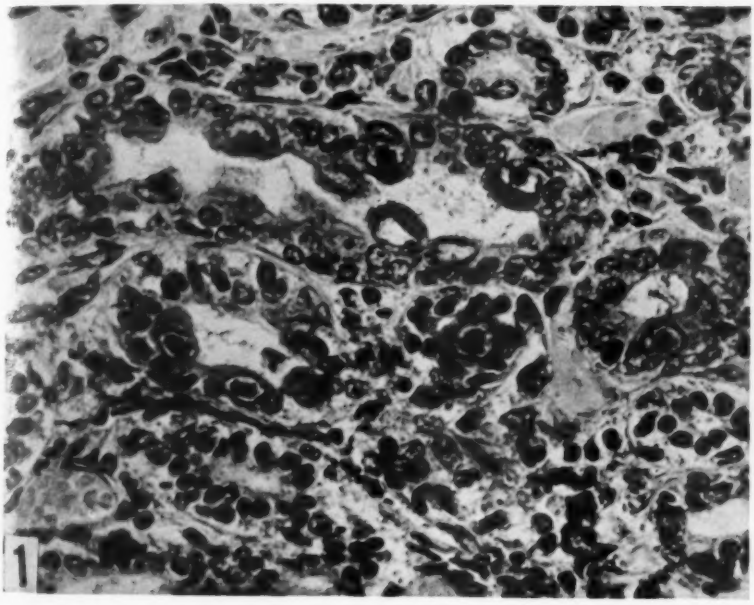
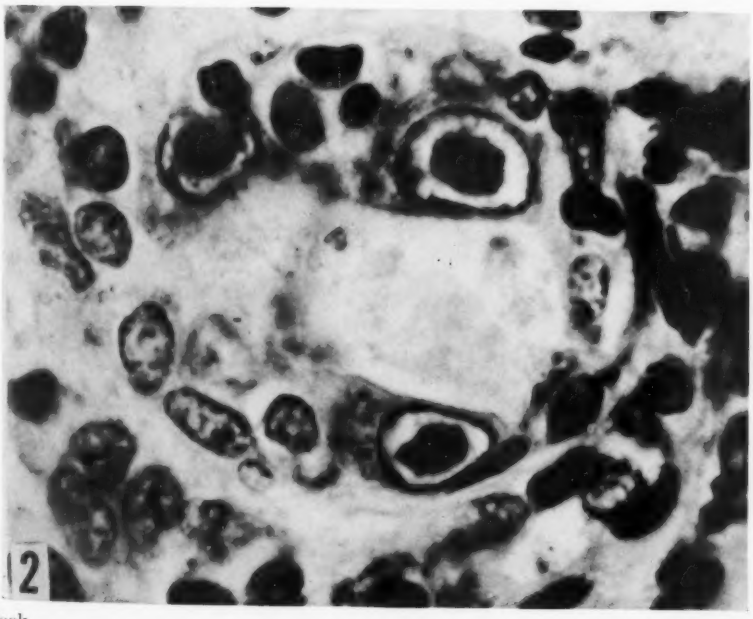
PLATE 80

FIGURE 1.—Salivary-gland virus: intranuclear inclusion bodies in a natural infection. × 235 (AFIP Access. No. 598633.)

FIGURE 2.—Intranuclear inclusion bodies in a tubule of a salivary gland in a natural infection. × 1800 (AFIP Access. No. 598633.)





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Infection in Laboratory Animals with Pleuropneumonia-like Organisms [1]

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The first member of the pleuropneumonia group was isolated in 1898 by Nocard, Roux, and their associates at the Pasteur Institute in France. The highly pleomorphic organism was demonstrated by the dark-field examination of pleural fluid from cattle infected with bovine pleuropneumonia. It was minutely described, cultivated *in vitro*, and given the now obsolete name *Asterococcus mycoides*. Subsequent work by many investigators showed that similar organisms were widely distributed in nature, both as parasites and saprophytes. The pleuropneumonia group now includes members from man, all of the domestic animals, chickens, turkeys, rats, and mice. Most of these organisms are host specific. In addition, free-living forms are found in soils, compost, and sewage. In 1935, Klieneberger-Noble introduced the term pleuropneumonia-like organism (PPLO), now in general use to designate the members of the group other than the original type species.

Rats and mice are the two laboratory animals that are particularly subject to PPLO infection and the present discussion will be limited to them. It is based on observations made in our laboratory and by other workers elsewhere (*1*). The limitation of space does not permit individual recognition.

Natural Infections in Mice

The respiratory tract.—PPLO invade the respiratory tract of mice by way of the nasal passages and produce a chronic disease which is generally known as infectious catarrh. It is by far the most prevalent PPLO infection in these animals. The disease is characterized by a persistent exudative inflammation of the nasal passages, the middle ears, and the lungs. The organisms enter the upper air passages by way of the nares. Active multiplication occurs on the nasal mucosa and is attended by local irritation. The host responds by producing a copious, semifluid, mucopurulent exudate. There is no actual discharge from the nares and exudate is detected only by exposing the nasal passages and aspirating them with a fine capillary pipette. A gram-stained film is made of the washings. If a true exudate is present, numerous polymorphonuclear leukocytes are found in all fields. PPLO may also be seen as small,

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1955.

Journal of the National Cancer Institute, Vol. 20, No. 5, May 1958
459041—58——9

spherical, or slightly elongated, gram-negative bodies. They may be extracellular or intracellular, within or on leukocytes and epithelial cells. In nasal exudates from long-standing cases of infectious catarrh, the microscopic recognition of PPLO is difficult.

From the nasal passages PPLO migrate to the middle ears, presumably by way of the eustachian tubes. Multiplication in this locality is also active and is again attended by a vigorous and persistent inflammation. The resulting exudate, rich in leukocytes, accumulates in the tympanic cavity and remains there throughout the life of the host. The exudate may be sufficiently copious to produce an outward bulge in the tympanic membrane, but actual rupture has not been observed. The middle-ear reaction may be either unilateral or bilateral. Extension to the inner ear may occur, with resulting circular movements, but is uncommon. Otitis media is generally detected only at autopsy by exposing the tympanic cavity and proceeding as with the nasal passages.

A state of equilibrium between the host and the invading PPLO is finally reached and may endure for a period of months. During this time the only outward sign of infection is an intermittent chattering sound, which has been given many names. In weanlings even this sign may be lacking. Otitis media amd rhinitis are commonly the only manifestations at autopsy. Once initiated, infectious catarrh persists and recovery is rare. PPLO may ultimately reach the lung by way of the trachea and a spreading bronchopneumonia results. Many infected animals will show no pulmonary involvement, however, at autopsy. The reaction in the lung is much the same as elsewhere. Irritation of the bronchial mucosa is attended by mucopurulent inflammation and the lumen becomes plugged with exudate, as indicated in figure 1. The pneumonia progresses slowly, but ultimately entire lobes are completely consolidated and airless. There is no reaction in the pleural cavity, except for an occasional adhesion. Deaths are sporadic and usually occur only in adults.

With advancing pneumonia, the mouse shows distinct outward signs of illness. The hair becomes roughened, and abraded areas may appear in the skin. The animal loses weight and has a lean, pinched appearance. This state may persist for a long time but not infrequently is terminated by death before the end of the natural life span is reached.

Other localities.—A particular type of PPLO may invade the conjunctivae of mice and produce a persistent low-grade inflammation. Large epithelial cells containing clusters of spherical organisms are found in films of the lacrimal exudate (fig. 3).

PPLO are occasionally carried to the central nervous system, with localization in the brain. They have been isolated from the brains of untreated and seemingly normal mice, but more commonly after the intracerebral injection of other pathogenic agents.

Experimental Infections in Mice

The chief manifestations of infectious catarrh are reproduced in normal disease-free mice by the nasal instillation of exudates and lung suspensions.

Chattering is usually detected by the 10th to the 14th day. Rhinitis, otitis media, and pneumonia are found in variable combinations at autopsy during the 4th week. PPLO are recoverable in culture from all 3 loci. If the observation period is extended, outward signs of illness appear and death commonly occurs. The interval between injection and death is highly variable and may occasionally extend to a year. The disease is regularly transmissible by direct contact, characteristic lesions appearing after 4 to 6 weeks of exposure. Transmission by indirect contact may occur, but is uncommon. Pure cultures of PPLO are infective on nasal instillation, but their activity is commonly less than that of exudates. The organisms continue to multiply in the nasal passages and middle ears but may fail to do so in the lung. The activity of cultures is further decreased by long-continued transfer.

Endemic pneumonia is appropriately considered here as a confusing factor in the diagnosis and transmission of infectious catarrh. This disease, sometimes called rodent bronchiectasis, is particularly prevalent in rats, but also occurs in mice. It is chronic, of viral etiology, and transmissible in both rats and mice by nasal instillation and contact. The most common manifestation is a slowly progressive bronchopneumonia. Otitis media also occurs in the mouse. The two diseases may coexist in mice and are difficult to separate.

Arthritis is produced in mice by some strains of PPLO on intraperitoneal, intravenous, or foot-pad injection of exudates and pure cultures. The wrists and knees are most often affected, but the toes may also be involved. Localization of the organisms in the joints is regularly attended by an infiltration of leukocytes and may be followed by necrosis of cartilage and a proliferation of connective-tissue cells resulting in ankylosis.

The conjunctival type of PPLO is readily transmissible by direct contact, though some variability occurs in the response of different mouse strains. The organisms are not readily established on the conjunctiva by the local instillation of lacrimal exudates or pure cultures.

The intraperitoneal injection of female mice with catarrhal exudates or pure cultures of the corresponding PPLO is commonly followed by inflammation of the fallopian tubes and the ovarian capsule (fig. 2). There is no similar reaction in the genital tract of the male.

The syndrome known as rolling disease is produced in mice by the intracerebral injection of PPLO, together with a neurotropic virus. Pure cultures of Sabin's type A (*2*), which elaborates an exotoxin, reproduce this condition in the absence of an added agent. Other types of PPLO may multiply in the brain but are not pathogenic as such.

Natural and Experimental Infection in Rats

Infectious catarrh is also found in the rat, with manifestations similar to those in mice. Cross infection occurs with PPLO from the two hosts. Involvement of the inner ear, resulting in labyrinthitis and twisting, is somewhat more common in rats than mice. Irritation of the respiratory tract is indicated by snuffling, and rales may be apparent if the lungs are

extensively involved. The disease is transmissible by the nasal instillation of exudates, lung suspensions, and pure cultures of PPLO. It is readily communicable by direct contact and to a much less extent by indirect exposure. The endemic pneumonia virus together with the presence of *Streptobacillus moniliformis* are confusing factors in the diagnosis and study of infectious catarrh in the rat.

Conjunctivitis, encephalitis, and salpingitis caused by PPLO have not been reported in the rat, but joint lesions are occasionally produced under natural conditions and may be initiated experimentally. The organisms are sometimes recoverable from abscesses and tumors.

Biological Characteristics of Rodent PPLO

Rodent PPLO closely resemble the pathogenic types from other animal species. The two outstanding characteristics are their extreme pleomorphism and their dependence on an accessory growth factor for cultivation. Morphologically, they vary in shape and size from minute spherical particles, about 0.3μ in diameter, to slender filaments, 100μ or more in length. Curved and hooked bacillary forms, indicated in figure 4, are peculiar to some strains. The smallest units are filterable. Attempts to integrate these divergent elements into a life cycle have been made by Klieneberger-Noble, Dienes, and others (*3–6*). The accessory substance for cultivation required by all the many rodent types is supplied by blood serum, ascitic fluid, and chick-embryo extract. In our laboratory, fluid or solid media (*p*H 8.0) are enriched with 20 percent horse serum. PPLO are sensitive to aureomycin and streptomycin, but resistant to penicillin. The addition of 2,500 units of the latter is useful in primary cultures to prevent contamination. Small colonies, rarely larger than 0.5 mm. that grow downward into the medium are produced on serum-agar plates. One form of PPLO colony (conjunctival type) is shown in figure 5 and, for comparison, a colony of *S. moniliformis* (fig. 6). In fluid media some types produce a slight turbidity, which show silk-like waves, some are finely granular, and others show no visual evidence of growth.

Distribution and Importance of Infectious Catarrh in Breeding Colonies

Infectious catarrh is one of the most prevalent microbial diseases of laboratory rats and mice. Most of the large breeding colonies are probably infected. The disease is commonly enzootic but may become epizootic, with a high morbidity rate. PPLO are readily transmitted from infected mothers to their nursing young by direct contact via the upper air passages. Transmission again occurs when the weanlings are brought together as stock animals. The cycle is completed by the chance selection of infected stock as breeders. Diseased animals are abnormal and their response to experimental manipulation may be altered. Accordingly, the recognition and control of infectious catarrh are essential measures in the maintenance of breeding colonies.

Summary

Organisms of the pleuropneumonia group (PPLO) are widely distributed in rats and mice and many different types are recognized. Infectious catarrh, a chronic disease that involves the nasal passages, middle ears, and lungs, is produced by PPLO in rodents. The disease is transmissible by direct contact and is prevalent in breeding colonies. Other organs in mice or rats, which PPLO may frequent naturally or experimentally, are the conjunctiva, the brain, the joints, and the female genitalia. Localization of the organisms is usually attended by an infiltration of leukocytes and exudation. PPLO are recoverable from all loci of involvement by cultivation on nutrient agar enriched with horse serum.

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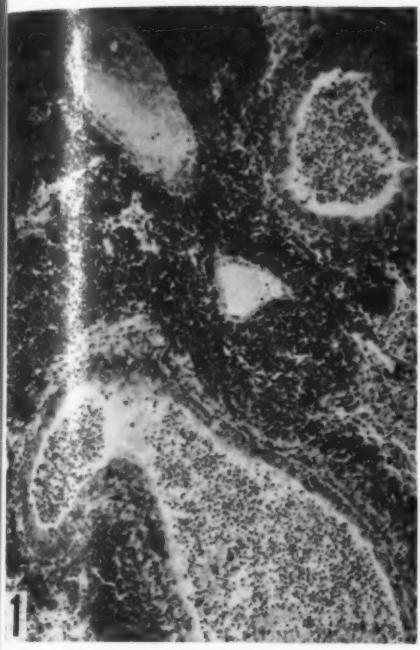
PLATE 81

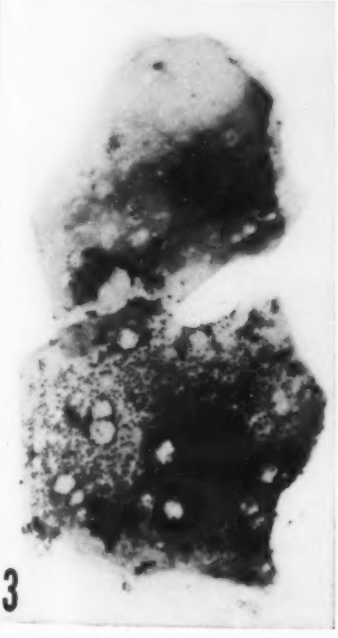
FIGURE 1.—Section of lung from a mouse with infectious catarrh. × 105 (Figs. 1, 2, and 3 *from* Nelson, J. B., J. Exper. Med. 65: 833, 1937; 91: 309, 1950; 100: 311, 1954.)

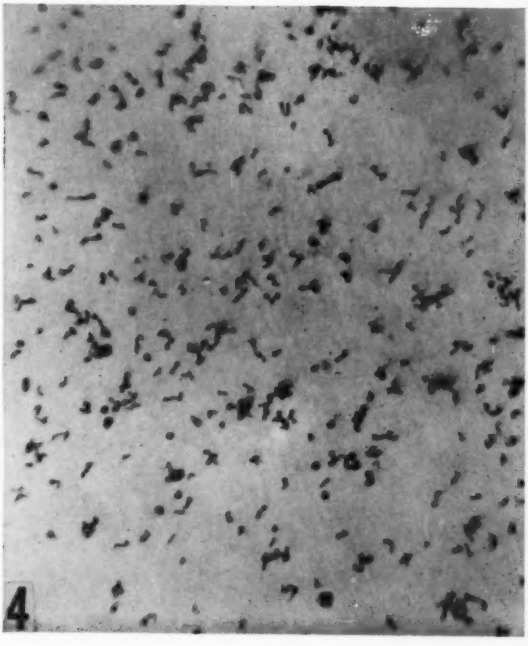
FIGURE 2.—Section of ovary and fallopian tubes from an infected mouse. × 58

FIGURE 3.—Film of lacrimal exudate from a mouse with conjunctivitis. × 1550

FIGURE 4.—Film of sedimented PPLO from a serum-bouillon culture. × 990





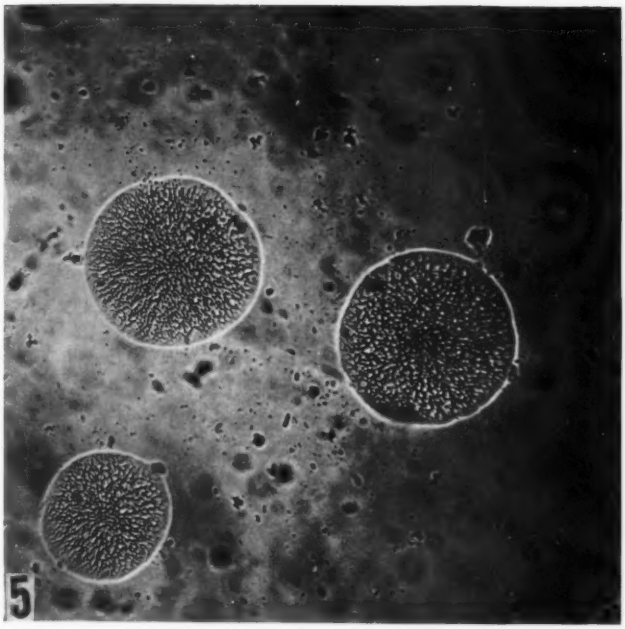


459041—58——10

PLATE 82

FIGURE 5.—Colonies of PPLO on a serum-agar plate. × 100

FIGURE 6.—Colony of *Streptobacillus moniliformis* on a serum-agar plate. × 100
(Fig. 6 *from* Nelson, J. B., J. Exper. Med. 91: 309, 1950.)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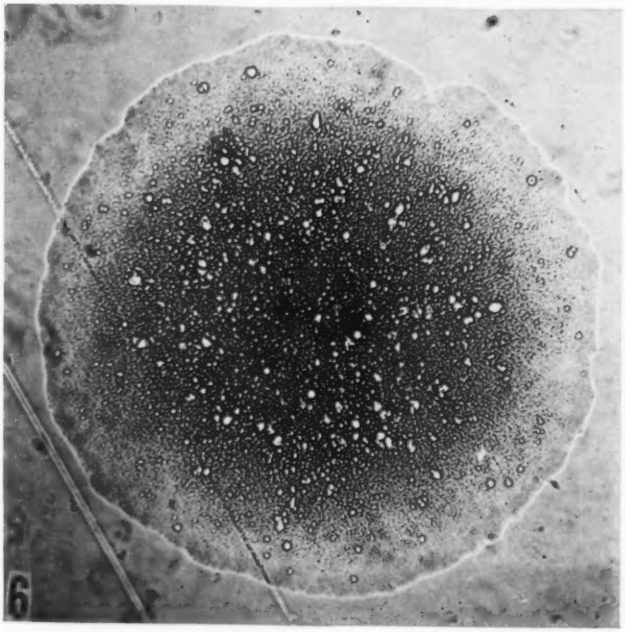
6

Myocarditis and Pulmonary Arteritis Associated with the Presence of Rickettsia-like Bodies in Polymorphonuclear Leukocytes [1]

ALWIN M. PAPPENHEIMER, *M.D.*,[2] *Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts*

This disease was discovered accidentally when a litter of 3-day-old sucklings were inoculated intracerebrally with brain suspensions from a moribund mouse. Examination of the dead mouse showed nothing of interest, neither in the half of the brain that was sectioned, nor in other viscera and tissues. One of the inoculated mice, killed for study on the 13th day, also failed to show any significant lesions. A blind passage was made into a second litter of 3-day-old mice. Two of these were killed on the 17th day, at which time they were small, hunched, rough-coated, and obviously sick, but not paralyzed. It was from these two animals that Mrs. Daniels and I first learned something of the nature of this disease, by finding rather remarkable lesions in the heart and lungs (*1*).

The disease proved to be difficult to study, because many individual mice seemed to be refractory to inoculation of presumably infective material, and one could never count on reproducing it with regularity. Furthermore, there were wide variations in the intensity of the lesions and, correspondingly, in the signs of illness during life, so that in many of the inoculated mice it was only by careful histologic study that a diagnosis could be established.

What made the lesions particularly interesting was the occurrence of numerous rickettsia-like bodies, chiefly in the cytoplasm of polymorphonuclear leukocytes. I shall recount our unsuccessful efforts to identify these bodies as true rickettsia.

In all, we examined some 200 mice, the majority inoculated with material suspected of containing the active agent, which included brain, heart, lungs, chick-embryo yolk sac, chorio-allantoic membrane, and chick tissue-culture suspensions. We tried all the usual routes of inoculation.

As controls, we had heart and lung sections from a much larger group of mice inoculated with known or suspected viral agents, and also normal mouse tissue and egg membranes. In none of these did we find a comparable type of myocarditis, or similar rickettsia-like bodies within leukocytes; and only two mice showed slight pulmonary arteritis such as characterized the disease under discussion.

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.
[2] Deceased.

Journal of the National Cancer Institute, Vol. 20, No. 5, May 1958
459041—58——11

Gross lesions of the heart were noted in only a few of the many mice examined. They consisted of circumscribed grayish patches on the surface of the ventricular muscle, and sometimes the auricles had a milky appearance. Usually there were no macroscopic lesions in the lungs, but a few of the mice that had been inoculated intranasally did show consolidated patches, usually in the upper lobes.

Microscopically, the heart was the seat of an acute interstitial myocarditis affecting both auricles and ventricles, and varying greatly in severity in regard to the distribution and extent of the cellular infiltrates (fig. 1). In some mice, only the auricles were affected, but usually lesions were present in ventricles as well. In the absence of serial sections, one could not establish any definite relation between the lesions and the blood vessels, but they did not appear to be primarily perivascular. Thus in the auricles, the inflammatory cells were often piled up beneath epicardium or endocardium. The valves were never affected, nor was there vascular thrombosis.

The cellular exudate was composed largely of well-preserved polymorphonuclears. Lymphocytes were not abundant, and no typical plasma cells were seen. The second component was large mononuclears, with vesicular nuclei and very prominent nucleoli. The cytoplasm of these cells was sometimes indistinct and not strongly basophilic. Whether the cells were histiocytes, young fibroblasts, or, possibly, myoblasts was not always easy to determine.

In contrast to the myocarditis produced by various strains of Coxsackie virus, there was never any marked degeneration or necrosis of the muscle fibers. The lesion was essentially an interstitial one.

A very unusual and distinctive feature of the disease was the involvement of many of the intrapulmonary branches of the pulmonary arteries (fig. 2), and, in some mice, of the main pulmonary artery itself (fig. 3). You will recall that in mice the pulmonary arteries are invested with striated-muscle fibers of the cardiac type—indeed, these muscular sheaths seem to be a direct extension of the cardiac muscle. In a large proportion of our infected mice, inflammatory lesions were present in and about the pulmonary arteries. These were in the form of more or less nodular accumulations of wandering cells, chiefly lymphocytes, but invariably included a fair proportion of polymorphonuclears, which were especially numerous near the arterial musculature, between or sometimes actually within the muscle cells. Necrosis of the fibers rarely occurred, the intimal elastic lamina was intact, there was no thrombus formation, and no secondary alteration in the pulmonary parenchyma.

Whereas, after intracerebral or intraperitoneal injection, the lesions were restricted to the arteries and their immediate vicinity, intranasal instillation produced a somewhat different picture, in that there were also patches of pneumonic consolidation with purulent exudate in bronchi and aveoli. Sometimes there was bronchiectasis with areas of collapse and emphysema. In other mice, only the arteritis was found.

Rickettsia-like bodies.—A clue to the possible etiology of these lesions

in heart and pulmonary arteries was afforded by the finding of innumerable minute coccobacillary bodies within the cytoplasm of the polymorphonuclears. They were best seen in sections stained with Giemsa and buffered with phosphate-citric acid in the proportions recommended by Lillie for various fixatives (fig. 4). In our preparations these bodies took a reddish color, which we did not succeed in changing to blue or purple by varying the proportion of alkali or acid buffer in the stain. In gram preparations, using safranine as counterstain, the bodies, though poorly stained, were definitely gram-negative. With Mallory's phosphotungstic acid-hematoxylin, after 24 hours' staining, they were sharply stained dark blue.

These were not found within other cell types—histiocytes, endothelium, or fibroblasts.

Impression smears from some of the pneumonic lesions showed brilliantly red, minute intracellular bacillary forms in Machiavello stained preparations (fig. 5). One could not be sure that these were the same bodies seen in the sections.

We attempted to secure more convincing evidence for the rickettsial nature of these bodies, which in size and morphology corresponded well enough with known rickettsial forms. That they stained red instead of bluish-purple with Giemsa was by no means typical, and we had no success with the Nyka methyl violet-metaniline yellow stain, which admirably brings out *R. prowazekii* and other rickettsia in tissue sections. On the other hand, the bodies in smear preparations did stain red with Machiavello like other known rickettsiae.

Their occurrence within polymorphonuclears almost exclusively, rather than within endothelial cells or histiocytes, might arouse suspicion as to their rickettsial nature, but this is by no means conclusive. In studying the pneumonic lesions from typhus cases collected in Cairo by the Typhus Commission (*2*), we were able to demonstrate rickettsia-like bodies in great number within the polymorphonuclears of the bronchopneumonic exudate, and similar observations have been made by others in the experimental pneumonia produced in rabbits or mice by intranasal instillation of known typhus material, so that the occurrence of these bodies within polymorphonuclears by no means rules out their rickettsial nature.

We tried to secure more positive evidence by inoculation into yolk sacs but completely failed to obtain morphologic evidence of their multiplication, even after 5 serial passages. However, the yolk-sac material, when inoculated into infant mice, again produced typical lesions in 18 out of 34 animals, an incidence higher than that obtained with brain suspensions. This at least suggests that some multiplication had occurred.

When yolk-sac material was dropped upon the chorio-allantoic membrane of 12-day-old chicks, edema and grayish nodular swellings along the vessels at the site of inoculation were found, after 5 days. Control membranes inoculated with normal egg yolk did not show these changes. Microscopically, the infected membranes showed epithelial proliferation, often with extensive necrosis and dense inflammatory edema and cellular infiltration of the underlying mesenchyma. Efforts to demonstrate the

rickettsia-like structures in Giemsa preparations were difficult because of the pleomorphic character of the leukocytic granules in chick embryos. It is well known that fowl leukocytes normally contain rodlike or discoid bodies as well as spherical granules. They are of various sizes and shapes, and some of them might easily be taken for the rickettsia-like bodies. More suggestive were phosphotungstic acid-hematoxylin preparations, in which only a few of the leukocytes contained minute, bluish-stained rods or granules; these may have been identical with the rickettsia-like bodies we were seeking, but one could not be sure.

Injection of these chorio-allantoic membranes into mice produced pulmonary arteritis in a third of the animals, so that the agent, whatever it was, seemed to be present in the lesions.

Injection of male guinea pigs with presumably infective mouse material caused no scrotal reaction or febrile response. Dr. Edward Murray, of the School of Public Health, Harvard University, carried out complement-fixation reactions with the serum of these guinea pigs against representative strains of typhus, Rocky Mountain spotted fever, and Q fever rickettsiae. All the tests were negative, nor could one obtain any specific serological reactions using the guinea-pig serum against antigens of infected mouse lung, yolk-sac, or chorio-allantoic membrane.

Although guinea pigs were refractory to this disease, we did obtain pulmonary arteritic lesions in several cotton rats inoculated intranasally, and the rickettsia-like structures could be found within the leukocytes. No myocarditis occurred.

Because of the morphologic resemblance of our rickettsia-like structures to the coccobacillary bodies in the nasal passages and lungs of mice, described by Nelson and regarded by him as belonging to the pleuro-pneumonia group, we endeavored, but without success, to cultivate pleuropneumonia microorganisms from our mice, using appropriate media.

In conclusion, we have only the morphologic resemblance of these structures to warrant interpreting them as rickettsia, and this is far from convincing.

There is one other observation. In sections of the skin from several of our infected mice, we found mites both on the surface and invading hair follicles, and these mites contained in the lining cells of their intestines minute coccobacillary bodies more or less like those in the lesions. We then examined our mouse colony and found it to be heavily infested with mites, which were identified for us by Dr. H. S. Fuller, of the Entomological Department of the Army Medical Service Graduate School, as *Mycoptes musculinus*, a member of the family Listrophoridae. Thinking that they might possibly be vectors of the disease, we ground up about 50 of these mites, and inoculated the suspension into mice. But we were disappointed in that we completely failed to transmit the disease in this manner.

I shall briefly discuss the question as to whether this disease is identical with the spontaneous myocarditis of mice described by other observers.

Adherents of the allergic theory of rheumatic fever have attempted to

produce rheumatic lesions in mice with repeated injections of egg white, and they found cardiac lesions in the uninoculated controls as often as in the injected mice. The lesions described differed from those in our disease because there were few or no polymorphonuclears, and the valves were often involved. If rickettsia-like bodies were present, they eluded observation, and there is no mention of pulmonary arterial lesions.

In our laboratory, lesions of the myocardium, often of great severity and extent, have been noted in mice inoculated with various strains of Coxsackie virus, and such lesions have been described also in infections with the EMC, Mengo, MM, and Columbia SK viruses. In these lesions there is massive necrosis of muscle fibers, absence of pulmonary arteritis, and no rickettsia-like bodies are demonstrable, so that we feel reasonably certain that we are dealing with a new and distinct disease. Whether it is really rickettsial in origin remains to be proved.

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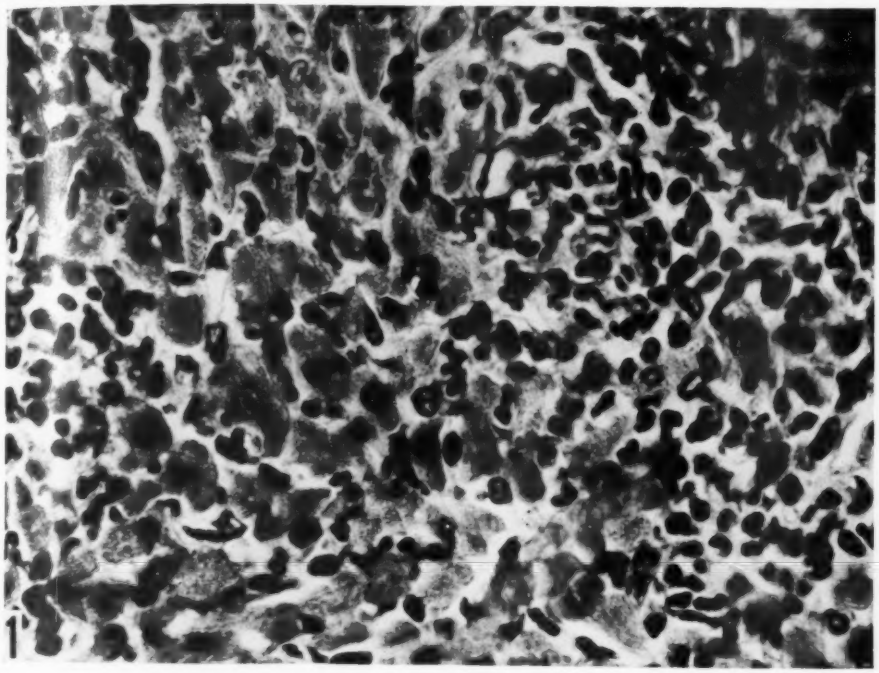
(*2*) PAPPENHEIMER, A. M.: Respiratory tract: Pathology of epidemic typhus. Arch. Path. 56: 415–418, 1953.

PLATE 83

FIGURE 1.—Three-day-old mouse inoculated intracerebrally and intraperitoneally with third-passage material, killed after 15 days. Acute interstitial myocarditis.

FIGURE 2.—Three-day-old mouse, killed 20 days after being inoculated intracerebrally and intraperitoneally with third-passage material. Acute pulmonary arteritis.



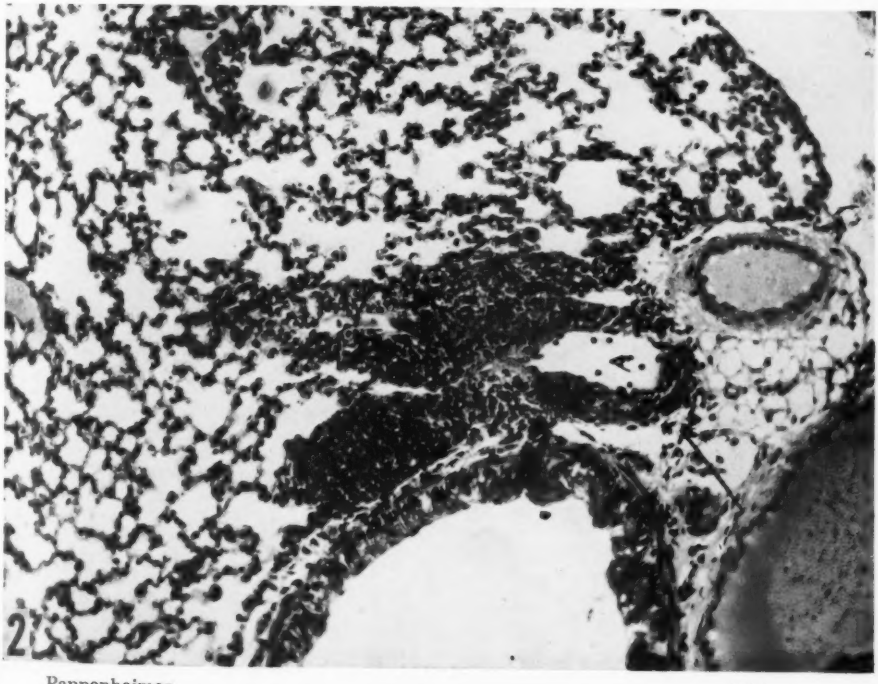
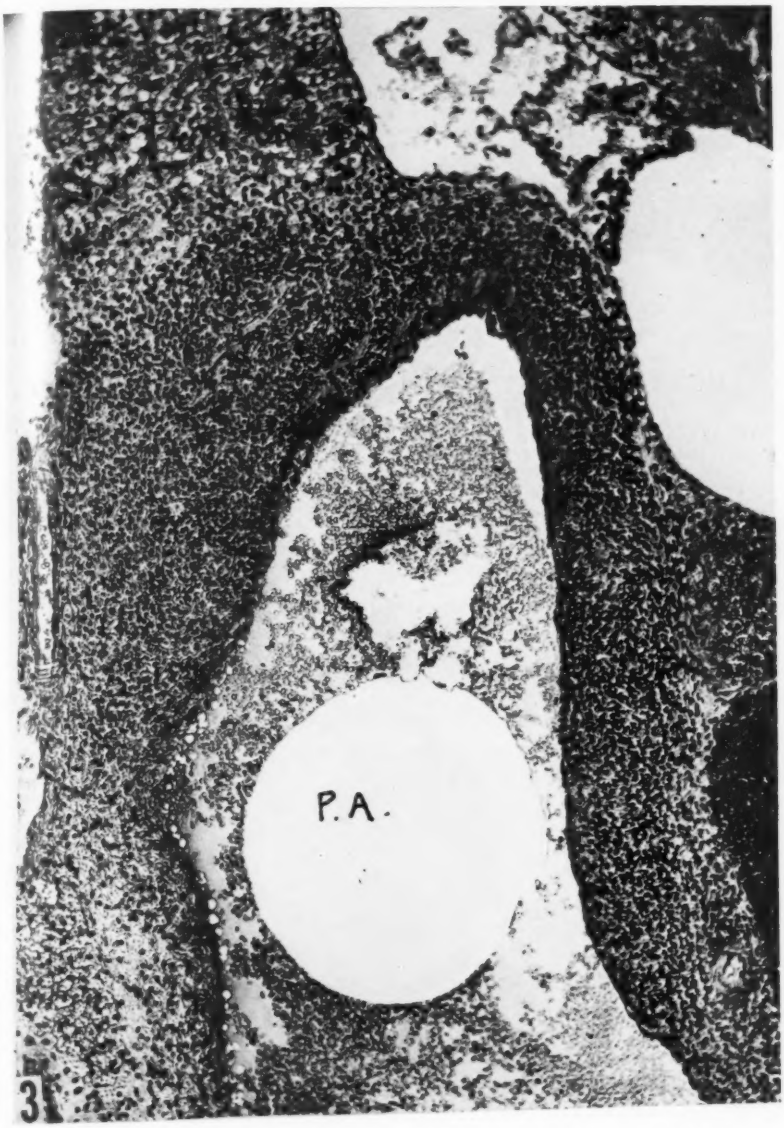


PLATE 84

FIGURE 3.—Three-day-old mouse inoculated intracerebrally with second-passage material 10^{-2}; killed 20 days later. Acute diffuse inflammation of main pulmonary artery (PA). (*From* J. Exper. Med. 98: 667–677, 1953, plate 61.)



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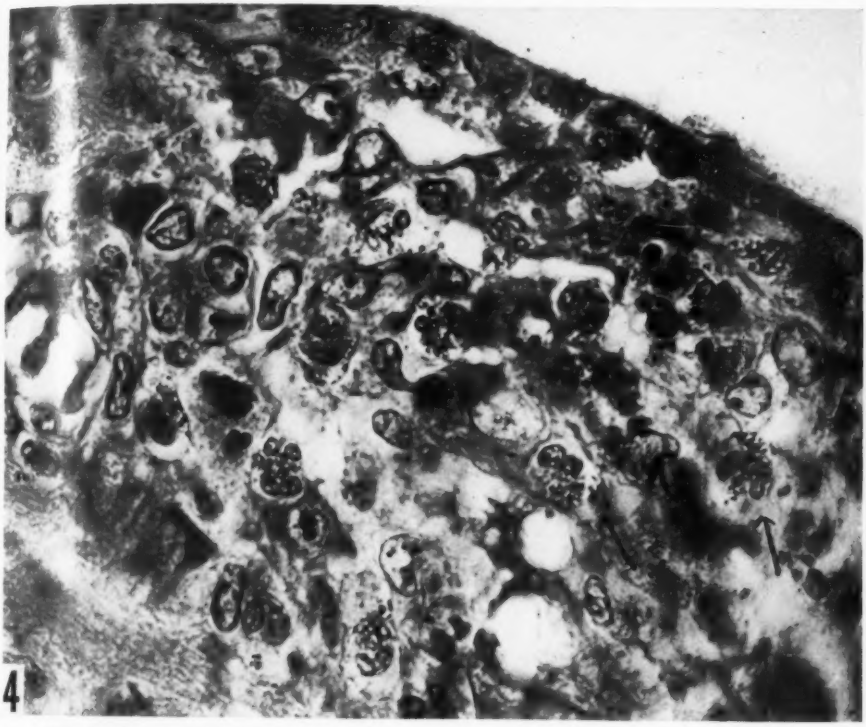
459041—58——12

PLATE 85

FIGURE 4.—Five-day-old mouse inoculated intracerebrally and intraperitoneally with third-passage material. Acute interstitial myocarditis. Rickettsia-like bodies in polymorphonuclear leukocytes. Giemsa stain. (*From* J. Exper. Med. 98: 667–677, 1953, plate 60.)

FIGURE 5.—Four-day-old mouse inoculated intranasally with heart-lung suspension of mouse that had received third-passage yolk-sac suspension. Impression preparation from pneumonic patch shows large numbers of red-stained rickettsia-like bodies, chiefly with polymorphonuclear leukocytes. Machiavello's stain. (*From* J. Exper. Med. 98: 667–677, 1953, plate 62.)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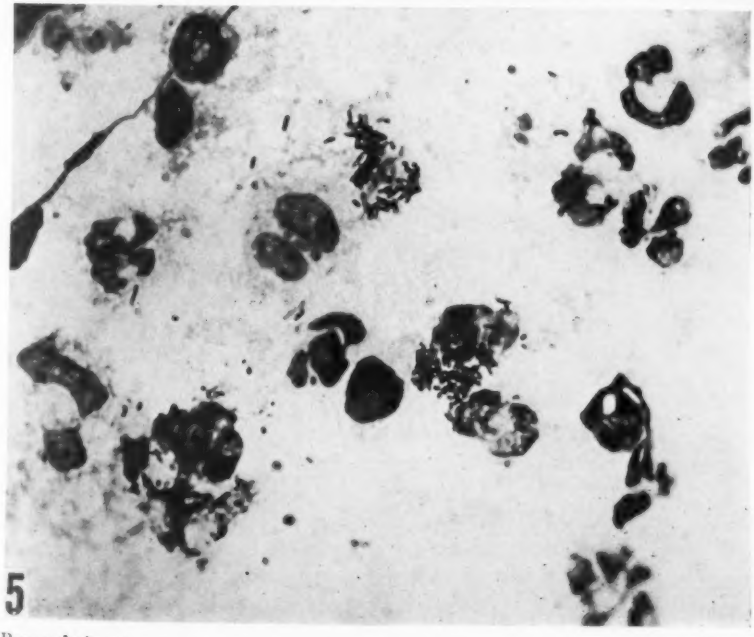
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Salmonellosis in Laboratory Animals [1]

ROBERT T. HABERMANN, *DVM, and* FLETCHER P. WILLIAMS, JR., *B.S., National Institute of Arthritis and Metabolic Diseases,*[2] *Bethesda, Maryland*

The first organism in the genus *Salmonella* was isolated, in 1855, by Smith and Salmon, from pigs that had died of hog cholera. They believed this organism to be the cause of the disease, and consequently it was given the name *Bacillus choleraesuis.* Subsequently, de Schweinitz and Dorset found this organism to be a secondary invader and one of the causes of infectious enteritis in pigs. In 1888, Gartner isolated another organism from a fatal case of gastroenteritis in a young man who had eaten raw meat taken from a diseased cow. The organism was named *Bacillus enteritidis.* In 1890 in a highly fatal epidemic in laboratory mice at the Hygienic Institute in Greifswald, Dr. Löeffler noted large, brownish-red spleens and small, yellow lesions in the livers of the dead mice. Groups of organisms in the capillaries reminded him of typhoid bacilli in human tissues. His studies of this bacillus showed that it was closely related to the colon-typhoid group and accordingly the name *Bacillus typhimurium* was proposed. The name *Salmonella* was proposed for the genus in 1900 in honor of Dr. Salmon.

Salmonellae are non-spore-forming, gram-negative rods closely related morphologically and physiologically to the other genera of the family Enterobacteriaceae. They are usually motile, though nonmotile forms occur; they attack carbohydrates, forming acid and, usually, gas; they do not ferment lactose, sucrose, or salicin, and do not form indol or liquefy gelatin. They are parasitic upon man and animals and usually produce inflammatory reactions in the intestinal tract.

At present there are about 150 species in the genus, of which 13 occur frequently and are widely distributed in man or animals or both. These organisms can be divided roughly into three groups: "Man adapted," such as *S. typhosa;* "animal adapted," *S. pullorum, S. abortusequi,* and *S. abortusovis;* and both "animal and man adapted," such as *S. choleraesuis, S. typhimurium, S. enteritidis,* and *S. anatum.*

The most important mouse pathogens, from the viewpoint of colony production, are *S. enteritidis* and *S. typhimurium.* Salmonella organisms are easily spread by intestinal discharges, and a single infected animal can easily contaminate a whole group. As both of these species are

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.
[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

Journal of the National Cancer Institute, Vol. 20, No. 5, May 1958
459041—58——13

widely distributed in many different hosts—mammal, reptile, bird, and even arthropod—the problem of preventing introduction into a mouse colony is a formidable one. There is practically nothing on which a rodent can live that at some level in production—on the farm, at the mill, in the freight cars—has not been in contact with salmonella carriers and possibly become contaminated. It is possible also for a colony of laboratory rodents to become infected by its human attendant. Mouse typhoid occurs so commonly in rodents that only with elaborate precautions can a colony be maintained free from infection. Animals suffering from the common form of disease may harbor and excrete the organisms for months, thus maintaining infection in the colony; or the infection may be introduced from without, in the food, or from wild rodents gaining access to the animal rooms. The incidence of carrier infection in stock mice may vary from 1 to 20 percent.

The naturally occurring or spontaneous salmonella infections are generally bacteremic diseases and may run acute, subacute, or chronic courses. The various bacilli produce essentially the same clinical picture. Infection, as a rule, takes place by the oral route and the incubation period extends from 3 to 6 or more days. The first sign of illness is loss of normal activity; the animal's hair becomes ruffled and loses its normal gloss; anorexia develops, though usually it is not complete, and loss of weight occurs. Later, conjunctivitis may develop, and the respiratory rate is accelerated. The feces are usually formed but are softer and lighter in color than usual. The temperature usually remains within normal limits, though a terminal rise and fall may occur.

In the acute form, the disease progresses rapidly and death may occur within a week. In the chronic form, there may be no signs of illness, or the disease may be a slowly progressive sickness until death. All degrees of disease between these extremes are met with, depending upon such variables as the exposure, resistance of the individual mouse or strain of mice, virulence of the organism, and external environmental factors such as temperature and diet.

Salmonellosis in the Mouse

The pathogenesis of salmonella infection in mice has been studied extensively, probably because of its similarity to human typhoid fever. After oral administration of the organisms there is a transitory excretion of bacilli in the feces. Invasion of the lymphatic system then occurs, with involvement of the intestinal lymph follicles, mesenteric lymph nodes, and, less often, the tracheal, bronchial, and cervical nodes. Here multiplication presumably takes place, bacteria are carried by lymphatic channels such as the thoracic duct to the bloodstream, and a transitory bacteremia ensues (2nd to 4th day), which is terminated by removal of the bacilli through action of the reticuloendothelial cells, particularly in the liver and spleen. Bacterial proliferation occurs in the lymph nodes, liver, and spleen for the next 2 to 4 days, as evidenced by the increase in the number of bacilli found in these organs, but the blood remains sterile.

Finally, a progressive reinvasion of the bloodstream results in generalization of the infection throughout the body and secondary invasion of the intestines. Bacilli are found in the tissues, gallbladder, bile, and urine after the septicemia has become established. The bloodstream is directly invaded only when the infecting strains are of the highest virulence and toxicity or when an overwhelming dose is given. Following the second bacteremia, the bacilli multiply rapidly in the intestines and may overwhelm the normal flora. In cases of chronic infection like that in mice which have survived natural or experimental infections, organisms persist in the spleen, liver, lymph nodes, and gallbladder for months and are intermittently or continuously discharged in the feces. Fatal infection may also be produced by applying bacilli to the depilated intact or lightly scarified skin, to the mucous membranes of the conjunctiva, or by inhalation.

The pathological changes seen in very acute infections are not characteristic but resemble those of any septicemic disease. Microscopically, the findings are those of hyperemia and congestion of all the organs, fatty degeneration in the liver, and severe catarrhal inflammation of the intestinal mucous membrane. Focal lesions are infrequently found in animals dying before the 5th day.

The role played by toxins, "lytic products," or specific substances derived from the bacteria is difficult to define, but presumably such products of the organisms account for the leukopenia and focal necrosis in the liver.

Animals living 1 or 2 weeks show more typical lesions. Emaciation is usually pronounced and the abdomen appears enlarged due to increase in the size of the liver and spleen and to intestinal distention. On necropsy the liver is enlarged and the spleen may extend down to the level of the pelvic bones. The intestinal serosa is usually reddened and injected, and the contents of the bowel vary from thin, yellowish-watery material containing mucus to soft or normal stool. In severe cases mucus appears in increasing amounts, and hemorrhages into the mucosa and lumen are found. Ulcerations occur and changes are found in the pyloric portion of the stomach. Microscopically, the picture is that of severe enteritis with capillary injection, denuding of the epithelium, ulceration of the follicles, and infiltration with polymorphonuclear leukocytes, monocytes, and large mononuclear cells. The mesenteric lymph nodes are hemorrhagic and often show focal necrosis.

The spleen is regularly enlarged to 3 or 4 times its normal size. It is dark red or reddish-purple; its capsule is tight and its consistency firm. On sectioning, the pulp protrudes and the cut surface is mottled with irregular, hemorrhagic, and gray areas. Microscopically there is congestive hyperemia, degeneration of the lymph follicles, and occasionally focal areas of necrosis.

The liver is markedly enlarged, varies from a deep red to a brownish-yellow, and is friable in consistency. Scattered yellow lesions of various sizes are seen. Microscopically the early lesions consist of foci of mono-

cytes, and later these foci increase in size and then become necrotic. The nearby liver cells also become necrotic but around these lesions the normal structural alignment is retained. The areas of focal necrosis show infiltration at the periphery with polymorphonuclear leukocytes and large mononuclear cells. Clumps of bacteria are often seen in the lesions.

The lungs may be entirely free from involvement or may show punctate hemorrhages or may be congested and hyperemic. Pneumonia sometimes occurs.

The bone marrow shows considerable damage, apparently caused by the depressant action of a toxic substance liberated from the bacilli.

Pathological changes in the remaining organs are inconstant. Occasionally, bacillary emboli are found in the glomerular tufts of the kidney. Focal inflammatory areas may be present in the myocardium. Bacilli are almost always seen in considerable numbers in smears of the blood, liver, spleen, and lymph nodes.

The organisms most commonly found in salmonellosis of the mouse are *S. typhimurium* and *S. enteritidis*.

Salmonellosis in the Rat

The appearance of the acute and the chronic type of salmonellosis is similar and resembles that described in the mouse. The incubation period is from 3 to 6 days or more. The *post-mortem* findings consist of friable, enlarged, congested liver with scattered, yellowish-gray foci, and congestion and marked enlargement of the spleen. Lymph nodes, especially the mediastinal and the mesenteric, are enlarged and soft, and the mesenteric lymph nodes often contain small cysts. In chronic cases the cecum is enlarged, distended, thickened, and inelastic. In the intestines, lymph follicles are enlarged, Peyer's patches are often ulcerated, and the mucosa is ulcerated and inflamed. Microscopically the changes in the lung may include small areas of pneumonia; the liver shows areas of focal necrosis, distention of the sinusoids with monocytes, with little or no alteration of the normal architecture. The spleen shows fragmentation of the lymphocytes, increased phagocytosis, and the changes in lymph nodes are like those in the spleen. In the intestines, denudation of the epithelium, areas of ulceration, and capillary injection are common, and occasionally there is ulceration of the lymph follicles.

Salmonellosis in the Guinea Pig

The clinical symptoms in the naturally occurring or spontaneous infection are like those in mice and rats. In experimental salmonellosis, when young guinea pigs, 18 to 23 days old, are offered 5 cc. of a 24-hour broth culture of *S. enteritidis*, the animals go off feed for 4 or 5 days after exposure and have temperatures of 104° C. or more for 3 to 6 days. The most prominent lesions found in these animals at necropsy occurred in the liver (fig. 3), spleen, mesenteric lymph nodes (fig. 4), and lungs. These

lesions bear close resemblance to those found in the rat except that hemorrhagic and ulcerative changes are present in the colon and cecum (figs. 2 and 5) of the gunea pig.

Salmonellosis in the Rabbit and Hamster

The symptoms and lesions produced by *S. enteritidis* in rabbits and hamsters are much the same as in the mouse, rat, and guinea pig. However, it appears that rabbits are more resistant to the disease.

Salmonellosis in Monkeys

Salmonella infection occurs in monkeys (fig. 1) and is especially serious in groups of monkeys that are crowded together and kept under unsanitary conditions, in shipped animals, and in animals whose diet is changed abruptly. The course of salmonella infection in monkeys is like that seen in other animals.

Salmonellosis in Dogs

The incidence of salmonellosis in dogs has been determined in recent surveys in Florida, Michigan, and other States. It appears from the literature that salmonellosis is not important in healthy dogs but may be pathogenic for dogs in a debilitated condition. Runnels (*1*) sums up salmonellosis in dogs as follows:

Acute enteritis of dogs, in kennels, veterinary hospitals, and dog-racing tracks in Florida and other parts of the country, is referred to as salmonellosis in the literature. Many mature, healthy dogs harbor various species of salmonella in the intestinal tract but do not become sick. In fact, such dogs do not develop enteritis when fed cultures of salmonella. Debilitated dogs, puppies, and distemper-infected dogs become victims of the infections and develop a severe, acute, catarrhal or hemorrhagic enteritis.

Isolation of Salmonella Species from Laboratory Animals

Different kinds or species of salmonella organisms have been isolated from a number of laboratory animals. *S. enteritidis* was isolated from guinea pigs, rats, mice, hamsters, dogs, and monkeys. *S. typhimurium* was isolated from rats, mice, rabbits, and monkeys. *S. florida* and *S. bredeney* were isolated from guinea pigs and rats. *S. illinois* and *S. newport* were isolated from rats. *S. poona* from guinea pigs. *S. tennessee* from mice and dogs. *S. senftenberg* and *S. california* from mice. *S. sandiego*, *S. bareilly*, and *S. stanley* from monkeys. *S. saintpaul*, *S. meleagridis*, *S. montevideo*, and *S. oranienburg* from dogs.

In a recent survey of salmonellosis in man and animals in Georgia, Stewart and De Capito (*2*) found that salmonella organisms were often isolated from man and animals and that the predominant types found in both were the same. They point out that the intimacy of contact between man, dogs, and cats makes it obvious that direct spread from animals to man or from man to animals can easily take place.

Composite Sample Technique

Fecal samples are collected from a clean sawdust pan for each animal in the cage. Where more than 4 rats or guinea pigs are present in a cage, the fecal samples are broken in half and half a sample is used in the test. The samples are placed in a centrifuge tube with 10 cc. of tap water and the tube is numbered according to the cage number. Samples from 150 to 300 cages are sampled at a time. The samples in the centrifuge tube are macerated with a wooden applicator and 3 or 4 cc. of the composite sample are poured into a sterile tube of selenite F enrichment broth. The selenite F enrichment-broth tubes are incubated for 18 hours and then streaked on 1 to 100,000 brilliant-green agar plates. These plates are incubated for 18 hours and then 4 to 5 salmonella-like colonies are picked and inoculated into triple-sugar-iron agar slants if agglutination tests are not run. However, typical salmonella colonies can be tested for *S. enteritidis* and *S. typhimurium* by the agglutination plate test using rabbit immune serum. If the agglutination tests are positive, indicating *S. enteritidis* or *S. typhimurium*, the samples are saved for confirmation by Dr. P. R. Edwards, of the Communicable Disease Center in Atlanta, Georgia. If the triple-sugar-iron slants show acid and gas in the butt with H_2S, the cultures are transferred to urea and indol media. If both of these tests are negative, then the cultures are run through the tinctorial sugars—sucrose, lactose, and glucose. If the cultures are sucrose- and lactose-negative and produce acid and gas in glucose, this indicates that the test organism is a *Salmonella* (*3*).

Further identification of the salmonella organism is made by the determination of the antigenic structure, either by cross absorption tests with known agglutinating antiserum or by agglutinations with antisera previously absorbed to remove all but specific antibodies. It might be mentioned at this point that the above composite sample technique and method for the isolation of salmonella was used in screening approximately 38,000 laboratory animals at the National Institutes of Health when the animal colonies were screened for infectious diseases before they were moved from the old animal buildings to the new animal quarters.

Cummings (*4*) suggests a shorter method for salmonella identification by sampling the drinking water from each cage of mice and plating about 1 cc. of the drinking water directly on brilliant-green agar plates or other suitable plate media. He points out that culturing drinking water is easier and quicker than culturing mouse feces, that there is less colony variation and more typical salmonella growth on the agar plates, and that primary inoculation into selenite F broth is an unnecessary procedure.

Treatment of Salmonellosis

The treatment of salmonella infections for the elimination or eradication of the infection from laboratory animals has been unsatisfactory. Slanetz (*5*), in 1946, reported on the treatment of *S. enteritidis* infection in 30 mice by offering 0.5 mg. of streptomycin in the drinking water per day.

The dosage was calculated from the average daily consumption per mouse of 5 to 7½ cc. of water. Streptomycin was administered to all the animals for 7 consecutive days. The 30 positive adult mice and 24 of their young were tested 4 weeks after the use of streptomycin and at monthly intervals thereafter. On the first test, one adult was positive but on all subsequent tests, adults and the young were negative for salmonella. This report indicates that such antibiotic therapy might be the means of clearing a colony of mice infected with *S. enteritidis* with the minimum amount of effort. However, in a later report, Dr. Slanetz stated that *S. typhimurium* was considerably more resistant to the streptomycin therapy. Seligmann and Wassermann (*6*) treated 20 gm. mice infected with *S. typhimurium* with chloramphenicol (Chloromycetin)® in the concentration of 1,200 mg. per kg. of body weight. The treatment prolonged the survival time of the treated mice, but on necropsy all of the mice were infected. Pöldre (*7*) treated 5 human cases with chlortetracycline (Aureomycin®) but the patients showed no improvement. The organisms disappeared after 3 to 4 days of treatment in 4 of the patients, but organisms were recovered in one case. Lutz (*8*) treated mice, infected by salmonella, with chloramphenicol and Puromycin®.[3] These two antibiotics did not completely eliminate the organisms from the intestines of mice. Korns and Albrecht (*9*) treated 12 chronic typhoid carriers harboring *S. typhosa* with oxytetracycline HCl (Terramycin hydrochloride®) for a 10-day period. The patients persisted in excreting the organism after the treatment. We have used nitrofurazone (Furacin®) in the feed (160 mg. per guinea pig), in the treatment of *S. enteritidis* infected animals, and Aureomycin in the feed (2½ mg. per mouse), in the treatment of *S. enteritidis* infected mice. Furacin was found to be toxic and ineffective in the treatment of the guinea pigs, and Aureomycin in the dosage given also proved to be ineffective in the elimination of *S. enteritidis* from the experimentally treated animals.

Attempts to evaluate immunization against this disease have chiefly been carried out in conjunction with studies of experimental epidemics in mice (*10–12*). In general, it may be said that the use of killed vaccines or bacteriophages (*13*) are not effective in preventing fatal infection (*14*) or in the spread of the disease when vaccinated and normal mice are in close contact.

Prevention of Salmonellosis

The animal rooms should be rodent-proof, light, and well ventilated, with regulation of temperature and humidity. The floors should be considered to be contaminated at all times and nothing placed thereon should be permitted to come in contact with cages, racks, tables, or other equipment of the room without sterilization. Animals that escape from the colony should be destroyed and should never be returned to the colony. Redistribution of animals within the colony is a dangerous practice. Pigeons, sparrows, and wild rodents have been shown to be reservoirs of

[3] Stylomycin. Antibiotic under study.

infection and all must be carefully controlled, especially in proximity to food storage. Flies have been repeatedly implicated as mechanical carriers and must be destroyed in the colony quarters. Food and bedding are best stored in metal containers, bins, or special rooms protected from vermin and stray rodents.

Quarantine measures should be taken in the event of an outbreak of disease in the general stock. At the first appearance of the disease, the room should be rigidly isolated and the diseased mice cared for only by attendants who have no contact with normal animals. The animals in the infected colony should be redistributed into the smallest possible number per cage unit. A specific death is then an indication for destruction of all the mice in that cage. If the disease is very extensive, it may be necessary to kill all the animals. In a number of the animals the carrier's state may be persistent and care must be taken in adding new susceptible mice or in augmenting the number of animals per unit. The feces or water bottles should be cultured for salmonella until the examination of a sufficient number of samples of apparently normal mice and of those that died sporadically reveal no evidence of the disease.

New mice should be kept in quarantine for at least 3 weeks, and then distributed in separate cages with 4 to 6 mice to a cage. Necropsy examinations, with cultures, should be made on all dead animals. If infection is recognized, the cage mates must be killed. Should no cause for death be found, the other animals in the cage should be watched for an additional 2 or 3 weeks.

The application of general preventive measures is the only satisfactory way, in the absence of specific prophylaxis or therapeutics, to prevent the introduction and spread of salmonellosis in a mouse colony. Success will depend to a large extent upon the strictness with which the control measures are maintained.

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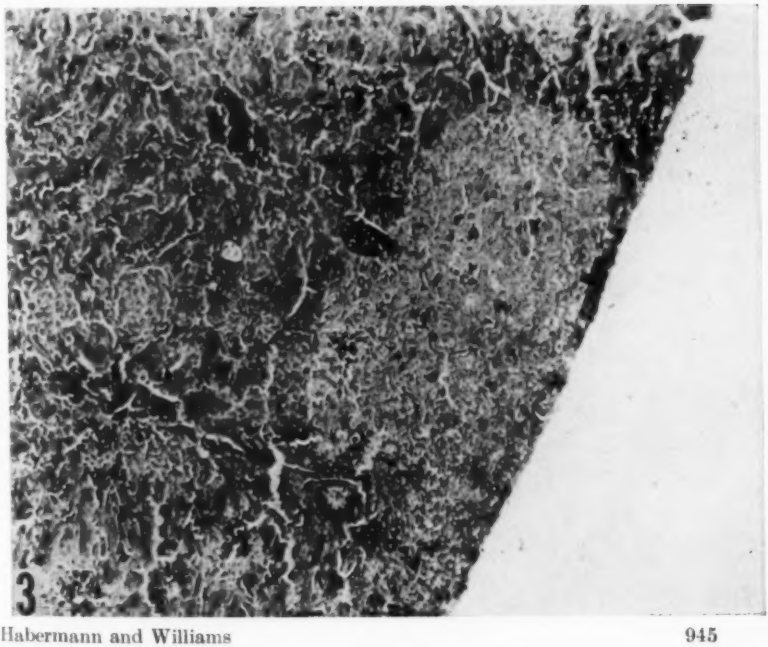
PLATE 86

FIGURE 1.—*Monkey salmonellosis:* ulceration and hemorrhage of colon with thickening of mucosa.

PLATE 87

FIGURE 2.—*Focal lesion* on the liver and ulceration of lymph follicle in the intestine.

FIGURE 3.—*Liver:* focal necrosis. × 125

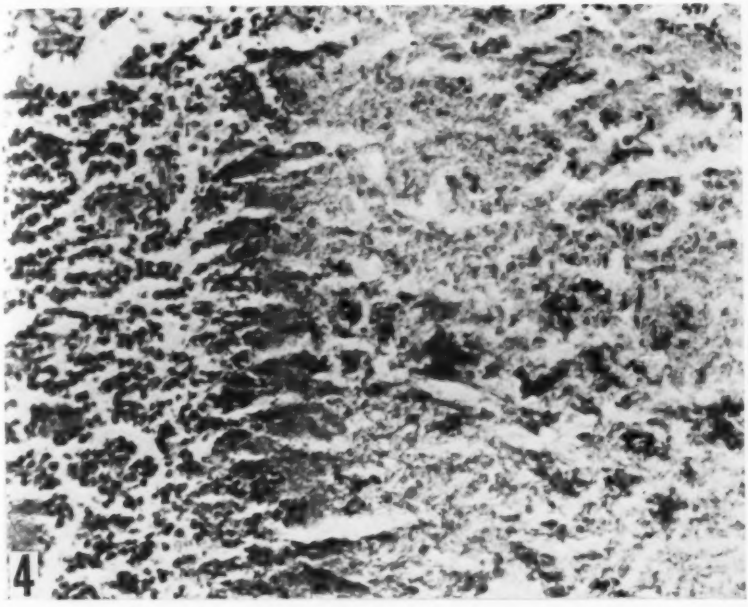


459041—58——14

PLATE 88

FIGURE 4.—*Salmonellosis:* mesenteric lymph node, focal necrosis. × 240

FIGURE 5.—*Colon:* necrosis of lymph follicle. × 90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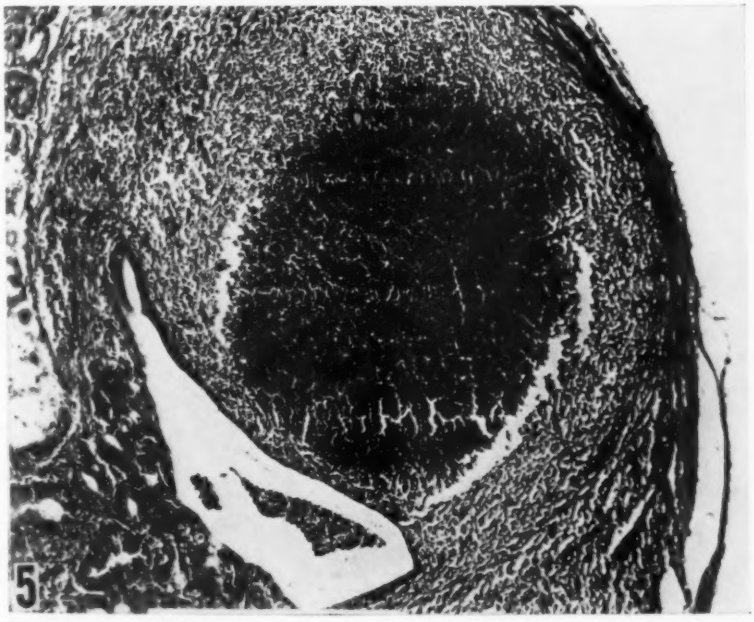
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Bartonellosis [1]

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The microorganisms commonly referred to as bartonella may be divided into two genera, *Bartonella* and *Haemobartonella*. *Bartonellae* are parasites of the erythrocytes, the reticuloendothelial system, and the vascular endothelium of man. The organisms are very polymorphous but are predominantly small rods and coccoids without differentiation into nucleus and cytoplasm. They are easily cultured on cell-free media. There is only one species in this genus, *Bartonella bacilliformis*. The disease produced by *Bartonellae* is limited to Peru, Colombia, and, perhaps, Central America, and has been known since the pre-Inca era.

The clinical disease may take several forms. Initially there may be an acute febrile anemia known as Oroya fever, with a mortality rate in untreated cases of about 40 percent. Survivors frequently develop transient benign vascular eruptions in the skin known as verruca peruviana. These two forms together constitute Carrión's disease. The anemia is caused by direct destruction of erythrocytes in the circulation, while the verrucose eruptions are vascular endothelial proliferations which contain bartonella organisms. Mixtures of these clinical forms occur, or they may occur singly. Relapses are usually of the verrucose form indicating the influence of an immune response. In endemic areas there are many asymptomatic latent infections.

Man and the vectors (Phlebotomus species) are the only spontaneous hosts for this organism. Experimentally, monkeys (*1*) and, occasionally, dogs (*2*) can be infected but only the verrucose form of the disease is produced. A complete description of this disease may be found in the excellent monograph by David Weinman (*3*).

The *Haemobartonellae*, in contrast to the *Bartonellae*, are natural parasites of erythrocytes, exhibit no demonstrable multiplication outside the blood, and do not produce cutaneous eruptions. Almost all the species are world-wide in distribution, rarely produce disease except in splenectomized animals, and are not easily cultured. They are markedly influenced by arsenotherapy, and are transmitted largely by arthropods.

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459041—58——15

Different species are distinguished on the basis of the host of origin, minor morphological variations of the organisms, and the susceptibility of other hosts to experimental infection. Twenty-two species have been described but some of these have not been well defined.

The type species is *Haemobartonella muris* [Mayer, 1921 (*2*)]. This species is of great interest to laboratory workers because albino rats are used frequently in experimentation and because bartonellosis is much more severe and has a higher mortality in the rat than in other laboratory animals. The disease in the rat has been reported from every continent and, while disease-free strains are also widely distributed, the infection is present in most laboratory colonies. Almost all adult rats in a colony may be infected. The disease is frequently not suspected, however, because it usually remains latent in the carrier animals unless activated by splenectomy.

A day or two after splenectomy there may be a slight increase in erythrocytes due to a relative anhydremia in normal as well as in carrier animals. The incubation period varies from 1 to 20 days or more following splenectomy, but it is usually about 3 to 5 days. Shortly before symptoms occur, there is a precipitous decrease in erythrocytes associated with the appearance of organisms in the blood (*4*). The erythrocyte count may fall 5 or 6 million per cu. mm. in 24 hours. The animals become quiet, anoretic, and rapidly lose weight. A 200-gm. rat may lose 40 gm. in a few days. There is a characteristic huddled appearance, roughened hair coat, pallor of feet, ears, and nose, and, frequently, hemoglobinuria. There may also be edema of the extremities and edema of the eyelids.

Examination of the blood reveals a very rapid sedimentation rate even after correction for the anemia. The hematocrit may be below 10 percent, the total erythrocytes below 1 million per $mm.^3$, and the hemoglobin 3 or 4 gm. per 100 cc. The mean corpuscular volume is increased to 125 μ^3 or more from a normal of about 61 μ^3. The mean corpuscular hemoglobin is also increased from a normal of about 17 $\mu\mu g$. to about 28 $\mu\mu g$., but the mean corpuscular hemoglobin concentration is decreased from 32 to about 23 percent. The anemia may be described as macrocytic and hypochromic. The leukocytes rapidly increase, starting even before the onset of anemia, up to 40,000 per $mm.^3$ or more. When very high values are reached, the increase is primarily in granulocytes.

Supravital preparations show a high percentage of reticulocytes, which frequently rise from a normal of less than 5 percent in adults to 40 or 60 percent. Polychromatophilic cells are present in large numbers. Metarubricytes are frequently seen and occasionally a few rubricytes. There is marked rouleaux formation and the blood often coagulates very rapidly after collection (*5*). There is marked anisocytosis and mild poikilocytosis. The erythrocytes have increased fragility and the indirect van den Bergh is positive.

Diagnosis is made by demonstrating the organisms in the blood, and it may also be necessary to inoculate susceptible splenectomized animals.

The organisms stain bluish with Wright's and Giemsa's stains and do not retain Gram's stain. Organisms are not seen in supravital preparations or in hematoxylin- and eosin-stained tissue sections. The organisms (fig. 1) are predominantly rods less than 1 μ long, frequently swollen on the ends, and may be curved or in chains. A variety of coccoid, diplococcoid, dumbell, filamentous, and other forms may occur. These organisms are almost all on or, perhaps, in the erythrocytes and there may be many organisms on almost every cell at the height of the disease. At the edge of a smear, organisms may be free in the plasma due to mechanical disruption of cells. Infective defibrinated rat blood can be preserved for at least 11 weeks at $-70°$ C. (*6*).

Symptoms last for a few days and then 40 to 80 percent of the animals die. The mortality approaches 100 percent when splenectomized rats are inoculated with organisms. Infection can be produced by almost any route of inoculation except oral. It is important to distinguish mortality figures among all splenectomized animals, splenectomized carriers, and inoculated splenectomized animals. Animals that recover may have relapses at intervals of several weeks. The only immunity is of the premunition type.

At autopsy the tissues are pale and may have an icteric tinge. There is a slight excess of clear yellow fluid in the abdominal and thoracic cavities. The mesentery is congested. The liver is rounded, friable, and slightly yellow. Microscopically there is centrolobular necrosis, mild, diffuse fatty changes, hemosiderosis and erythrophagia by the Kupffer cells. The kidneys are dark and swollen and microscopically there is severe tubular degeneration and cast formation in the lower nephron (*7*). In many acute cases there is tubular hyaline droplet degeneration similar to that seen in acute hemolytic crises. The tubule cells also contain granular iron-containing pigment. The bladder is distended with dark-purple urine when marked hemoglobinuria occurs as it does in over half the cases. The urine contains hyaline, granular, and blood casts, desquamated epithelia, blood elements, and large amounts of protein. The bone marrow is red, almost watery, and has a low myeloid-erythroid ratio. If there were predisposing factors other than splenectomy and the spleen were present, it would be soft, dark, and enlarged 2 or 3 times normal size. Microscopically the pulp is distended with blood. There may be mild reticuloendothelial and lymphatic hyperplasia. Hemosiderosis may be marked. There is erythrophagia here and in the lymph nodes.

In general, the symptoms and lesions are those one might expect to find in acute hemolytic anemia. The possible presence of these organisms must be considered not only in investigations involving the blood and hematopoietic tissues but also in experimental procedures, such as deficiency diets, blockade of reticuloendothelial system, experimental infections, tumor grafts, irradiation, or parabiosis.

Differentiation must be made from *Grahamella* (*8*) which are almost entirely long, slender rods, non-pathogenic, and easily cultured on cell-

free media (*9*). *Haemobartonella* is cultured with difficulty, and the media of choice is semisolid serum or blood agar such as Noguchi's leptospira agar (*10–12*). When *Haemobartonella* is easily cultured it is usually *Grahamella*. Eperythrozoonosis (*8*) is similar to bartonellosis in that splenectomy activates latent infection, a mild anemia is produced, and the organisms have not been cultivated and are susceptible to arsenotherapy. The organisms are predominantly ring-forms and differ from *Anaplasma* in having a clear central area. Diagnosis is made by the morphological distinction and animal protection tests. Mixed infections of *Haemobartonella* and *Eperythrozoon* together with the presence of *Grahamella* are a common occurrence in rodents, especially in mice (*13*).

It is possible to obtain bartonella-free rats commercially (Sprague-Dawley), or one can start a disease-free colony by treating the breeding stock, controlling ectoparasites, which are the most important source of transmission, and maintaining strict isolation and sanitation. Treatment is given "to effect" sterility but it is usually obtained with one or two injections of neoarsphenamine (*14*), 10 mg. per kg. intraperitoneally, or 1,000 to 10,000 units of penicillin per rat subcutaneously. Chlortetracycline and oxytetracycline are also effective (*15*), while sulfa drugs have no value.

H. muris is infective for most rodents. In general, a species of *Bartonella* isolated from a rodent is infective for other rodents but is not infective for the common domestic animals and primates.

Bartonellosis in the mouse is apparently caused by the same organism, *H. muris*. The disease is like that in the rat except the incidence within a colony is not so high, the anemia produced is usually very mild, there is no hemogolobinuria, the mortality is very low, and splenectomy is not always necessary to produce the disease.

H. tyzzeri, the cause of bartonellosis in guinea pigs (*16*), has not been reported in the United States. However, guinea-pig erythrocytes normally contain inclusions, which are probably defects in the formation of the cells, and which resemble *Haemobartonella* organisms.

Bartonellosis is rare in hamsters. The organisms resemble *H. muris*. No *Haemobartonella* organisms have been described in rabbits.

In recent years feline bartonellosis (*H. felis*) has been recognized (*17*). Splenectomy is not necessary for clinical disease to appear, and the anemia is very seldom fatal even when untreated. The organisms are primarily very small coccoid forms.

Bartonellosis occurs in dogs (*H. canis*) but only a dozen or more cases have been reported (*18–20*). Splenectomy is essential to produce the disease. *H. canis* has been transmitted to the cat.

In cattle, both bartonellosis (*H. bovis 21, 22*) and eperythrozoonosis are very common and produce little or no anemia. They are constant complications in studies of anaplasmosis, theileriasis, and babesiasis.

Summary

The microorganisms commonly referred to as bartonella may be divided into two genera, *Bartonella* and *Haemobartonella*. The *Bartonellae* are

parasites of the erythrocytes and fixed tissue cells of man. The *Haemobartonellae* are parasites of the erythrocytes but not of other tissue cells, occurring naturally in vertebrates other than man, and transmitted by arthropods. The organisms are very polymorphous but are predominantly rod or coccoid forms showing no differentiation into nucleus and cytoplasm. Almost all species of this genus are world-wide in distribution but rarely produce disease in animals without splenectomy. Bartonellosis is most severe in the rat in which an acute hemolytic anemia is produced by splenectomy of carrier animals.

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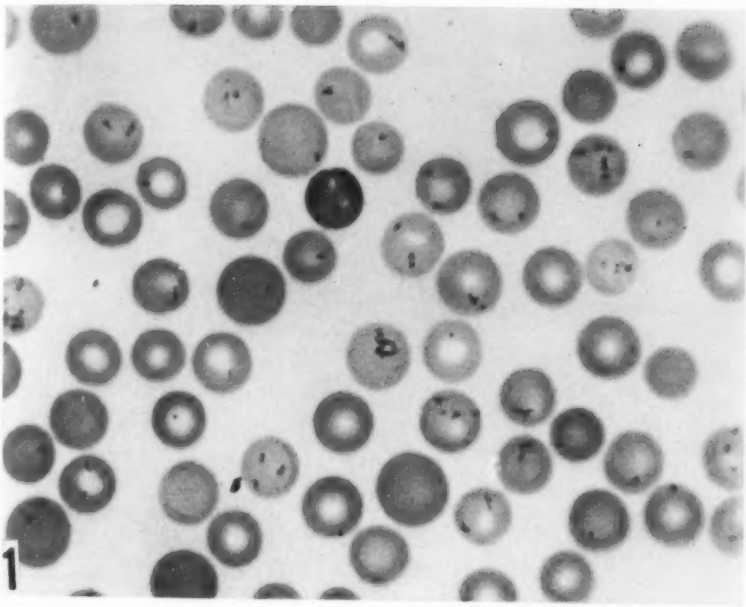
PLATE 89

FIGURE 1.—*Haemobartonella muris* in a blood smear. Giemsa stain. × 1320 (AFIP Access. No. 714216.)



Griesemer

Encephalitozoon Infection in Laboratory Animals [1]

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Encephalitozoon infection is a mild, chronic, inflammatory condition in rabbits. This disease was first described in 1922 by Wright and Craighead (*1*) as the cause of spontaneous paralysis in rabbits. A report by Levaditi and others (*2*) in 1923 proposed the name *Encephalitozoon cuniculi*, and reports by Oliver (*3*), Goodpasture (*4*), Cowdry and Nicholson (*5*), Smith and Florence (*6*), and others, described the organism and established the disease as a distinct entity. The German literature between 1918 and 1924 contains many reports on a disease in rabbits which must have been caused by *E. cuniculi* (*7*). In 1942, Olafson and Monlux (*8*) discussed the disease but thought that it was caused by a form of *Toxoplasma*.

The infection is observed clinically in rabbits, mice, guinea pigs, rats, and, occasionally, dogs (*9*, *10*). It is a mild chronic disease without distinct clinical diagnostic signs. Severe infections in the rabbit tend to cause roughening of the hair coat and may possibly stunt growth and induce limited depression. However, the disease cannot be positively diagnosed in the live animal. The blood picture is normal in mild cases but a slight lymphocytosis may accompany severe infections. Neurologic signs are generally absent, even though large areas of the cerebrum, midbrain, and pons may be affected, but some severely infected animals tend to show poor coordination and may occasionally withdraw when touched on the back.

The disease is mildly contagious. It is apparently a colony infection, and in colonies where a few animals exhibit histologic evidence of the disease, many will be found later to harbor the lesions. This is, however, not completely consistent. It is known that in a single pen of several rabbits one animal may be severely infected, while the others remain clinically and histologically free of the disease. Albino rabbits appear to be more susceptible to the infection than other breeds. Robinson (*11*), in a study at Great Lakes Naval Training Station Hospital on over 900 rabbits, observed the disease in at least 300, among which the percentage

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Journal of the National Cancer Institute, Vol. 20, No. 5, May 1958

459041—58——16

of infection in white rabbits was about twice that in colored breeds. A mild suppurative parametritis is seen in a few females, and organisms can usually be found in the urine of affected animals. This suggests a possible venereal origin of infection. The disease occasionally causes a mild myocarditis manifested by a limited periarteritis.

The outstanding feature of the disease is chronic nephritis characterized grossly by small cortical scars. Histologically these appear as depressions in the subcapsular area and are V-shaped, descending in some instances all the way to the corticomedullary border. The changes in these scars are principally those of replacement by connective tissue, with more or less complete obliteration of tubular structures and occasionally of glomerular tufts. Limited numbers of leukocytes are observed in these areas of scarring. Focal lymphoid infiltrates are found in both the medulla and cortex, but chiefly in the papillae of the medulla. In such areas, inflammatory foci tend to be small and to contain some necrotic tubular epithelium. Affected areas often display granular and homogeneous eosinophilic casts. Surprisingly, even in cases in which large areas of kidney are affected, uremia is not apparent, nor are changes observed in the bladder or ureters (fig. 1).

In the liver, when infection is severe, there are limited periportal infiltrates and occasionally small foci of lymphocytes are completely divorced from the hepatic triads.

The most striking lesions, consisting of typical granulomas with occasional giant cells, are observed in the brain. They are found in the cerebrum in both white and gray matter, in the midbrain, pons, and, very occasionally, in the medulla. In fatal cases the lesions sometimes are massive, but usually they are small, focal, and widely dissociated, and seldom cause widespread damage (fig. 2). The cerebellum and the spinal cord are rarely affected, though occasionally lymphocytic infiltrates are observed in these tissues. The meninges, usually those closely associated with areas of the brain where there are typical lesions, are occasionally lightly infiltrated with leukocytes. In affected areas in the brain there is occasionally some nerve-tissue degeneration. Perivascular cuffing, a nonspecific reaction in the brain to almost any type of pathologic insult, is observed rather routinely in these cases. Limited lymphocytic infiltrates are observed also in tissues remote from vessels. In many of the areas in the brain where the typical lesions are observed, there is an increase of cellularity in the walls of the blood vessels. The characteristic granuloma has a necrotic center in which one can usually find the typical organism and an external rim of histiocytic response, with epithelioid proliferation and limited leukocytic infiltrates.

In a few of the more severe cases, inflammatory infiltrates are observed in the myocardium, occasionally assuming rather large proportions.

The typical organism of *E. cuniculi* is a small, plump rod with rounded ends measuring approximately 0.7 to 1.0 μ in width by 1.5 to 2.0 μ in length. The rods vary from plump ovoid forms to elongated types and occur singly, in clumps, or in large numbers within pseudocysts. The

organisms have a clearly defined nucleus, with a dark-staining central band and rounded, pale areas at the poles (figs. 3 and 4). They are gram-positive and can be found either in histiocytic cells or extracellularly in the tissues. Very little additional information is available concerning *E. cuniculi*. It has not yet been cultured except in living animals, and then with success in only 50 percent of attempted transmissions, nor is it particularly host-specific since infective urine from a rabbit can be used to infect mice, guinea pigs, and rats.

One of the difficulties most frequently encountered in *E. cuniculi* infection is the necessity to differentiate between this organism and *Toxoplasma*. There are, however, several rather significant differences between the two diseases. Toxoplasmosis is usually a more acute disease and will readily cause death when introduced into mice, whereas Encephalitozoon produces only a mild chronic infection. *Toxoplasma* stains reasonably well with hematoxylin and eosin, while *Encephalitozoon* tends to appear fuzzy and indistinct. On examination under oil, the cytoplasm of *Toxoplasma* is easily recognized and usually tends to be distinctly granular; in contrast, *Encephalitozoon* contains no distinct cytoplasm and the organism generally has a smooth homogeneous appearance. In addition, *Toxoplasma* is consistently larger than *Encephalitozoon*. *Encephalitozoon* stains readily with silver impregnation, whereas *Toxoplasma* does not. Finally, although granulomas in nerve tissues are occasionally observed in toxoplasmosis, the granuloma noted in Encephalitozoon infections is characteristic.

Summary

Encephalitozoon cuniculi infection in laboratory animals is a mild, febrile disease. The disease is seen in rabbits, mice, guinea pigs, rats, and, occasionally, dogs. Organisms isolated in these cases are bipolar, ovoid to rodlike, gram-positive, and measure approximately 1.0 μ in width by 2.0 μ in length. They are usually observed in the epithelial cells of the papillae of the kidney, can be found in the urine of infected animals, and are noted in, and isolated from, the granulomatous lesions in the brain. The lesions produced include meningoencephalitis with granulomas in the cerebrum, midbrain, and medulla. The predominant inflammatory cells in all parts of the body are lymphocytic in origin. The meninges show patchy thickening, and infiltration by lymphocytes and a few plasma cells. Organisms are found singly and in clumps in granulomatous foci, in pseudocysts, and in nerve tissue, with no associated inflammatory response. In the kidney, interstitial foci of lymphocytes are observed principally in the medulla and occasionally in the cortex, along with some dilatation of the loops of Henle. Small subcapsular scars are found occasionally in the kidney and the organisms are present in the epithelial cells of the tubules in the regions of inflammation. Encephalitozoon infection may be distinguished from toxoplasmosis by the degree of illness, the morphology of the organisms, selected staining techniques, and characteristic lesions.

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Plate 90

Figure 1.—Kidney showing small subcapsular scars. Hematoxylin and eosin. × 135

Figure 2.—Cerebrum with granuloma. Hematoxylin and eosin. × 60

1

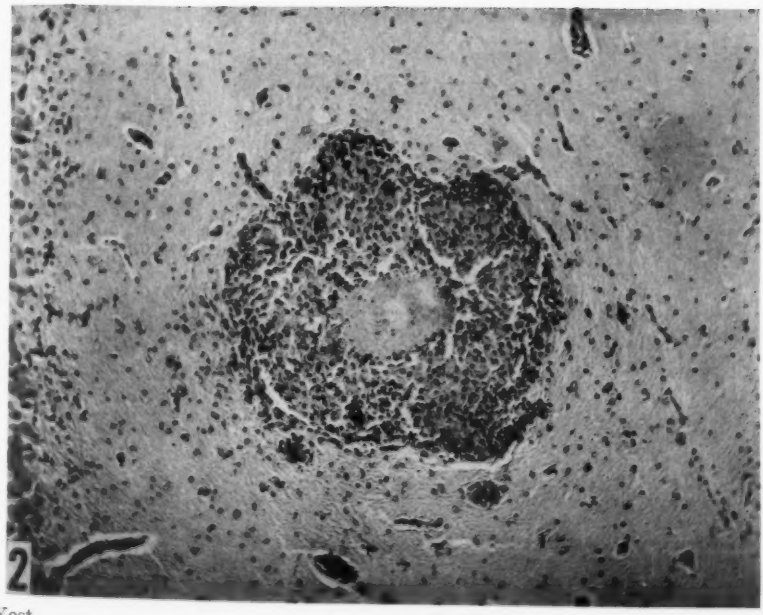
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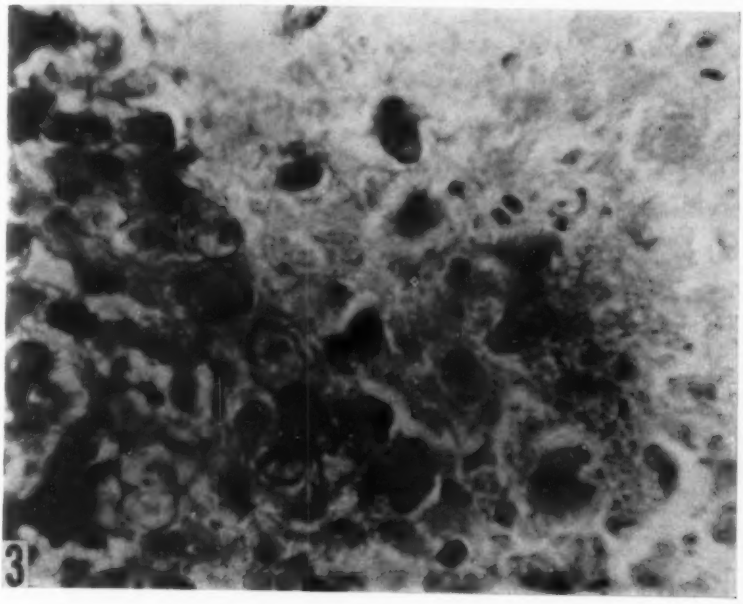
PLATE 91

FIGURE 3.—Granuloma in cerebrum, *Encephalitozoon cuniculi:* Goodpasture and Burnett stain. × 1700

FIGURE 4.—*Encephalitozoon* in cerebrum. Wright's stain. × 1700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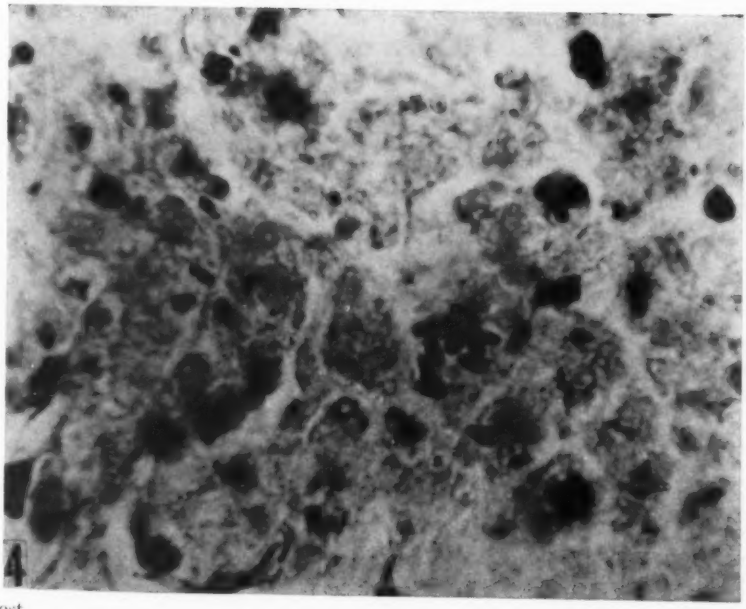
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Joint Diseases of Laboratory Animals[1]

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The present discussion is confined to small animals—mice, rats, guinea pigs, hamsters, and rabbits. The joints of these species have structural differences from those of man which may influence the comparative pattern of articular disease. In rats and mice, the epiphyses of the vertebrae and most of the large peripheral joints, except the elbow and distal tibia and fibula, do not close. In rabbits, except for the distal radius and ulna, proximal fibula, and lumbar vertebrae, they are fused by 10 months of age. In adult hamsters, the epiphyses of the knee remain open, as in rats and mice, but the capital epiphysis of the femur does not. The pattern in the guinea pig is like that in mice and rats. Another special feature is ossification of the menisci of the knee.

Degenerative Joint Disease—Osteoarthritis

Until recently, degenerative joint disease was not believed to occur in rodents (*1*). The Silberbergs (*2*) were the first to observe spontaneous osteoarthritis in mice. The changes are best developed in the knee. Usually they are of microscopic proportion only (fig. 1). In moderately advanced instances, however, there is a gross, though slight, deformity of the knee; this involves a varus angulation with medial displacement of the patella. In papain-digested skeletons prepared by Dr. M. Potter, we have seen marginal osteophytes about the knee and hip. The process appears to be initiated by necrosis of chondrocytes in segments of the articular cartilages. The loss of the articular cartilage is not accomplished by progressive fibrillation and erosion, which predominates in man, but by traumatic separation at the zone of calcification of the cartilage. Sclerosis of underlying bone, fibrillation of the cruciate ligaments, and fibrosis of the synovial membrane also occur. In advanced lesions, posterior subluxation of the femur on tibia may be seen. The lesion is more common in male than female mice and usually does not

[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.
[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

appear before 15 months of age. Genetic factors are involved, but nutritional and endocrine influences also operate (*3*).

Rats are highly resistant to the development of osteoarthritis of the knee, of the type seen in mice. In 157 old rats of 8 strains from the inbred colony of Dr. George Jay, Jr., observed by us, there have been only 3 instances of a similar lesion. A more frequent change, however, is cystic degeneration of the articular cartilages of the knee. This has its inception in a focus of chondromucoid softening, apparently beginning in relation to a deep lacuna in the articular cartilage (fig. 2). The chondromucoid stage is apparently short, because the great majority of the cysts appear to be empty. A minute point of perforation into the joint space usually can be seen (fig. 3), and it is presumed that the contents of the cyst have been discharged into the articular cavity. Despite this fact, the lesion does not progress to articular erosion. Small, mucoid resorption cysts are found frequently in the epiphyses of the tibia and femur in the region of insertion of the cruciate ligaments. These may be encapsulated by fibrous tissue or bone. Fibrillation and minor erosion of the articular cartilages of the tibiotarsal and talocalcaneal joints are commonly seen. These changes occur in the regions adjoining capsular attachments where a loss of viability of the chondrocytes takes place with regularity. In a small proportion of animals, this progresses to a degree of osteoarthritis like that described in the mouse knee.

Hamsters also are susceptible to spontaneous osteoarthritis like that described in the mouse, but it is infrequent before 2 years of age.

Guinea pigs may have mild degenerative disease after the 3rd year. We have had little experience with rabbits thus far but have seen severe osteoarthritis in the shoulders of an animal 3 years old.

Infectious Arthritis

Infectious arthritis seldom occurs as a sporadic lesion, though a number of epizootics have been reported. In general, it may be stated that when animals sustain bacteremia, which is not so fulminating as to cause rapid death, arthritis may result. Seeding of organisms to the synovial membrane, periarticular soft tissues, and the bone marrow may take place. If the joint involved is one in which the epiphyses have not united, the seeding may involve the metaphysis rather than the epiphysis. In the latter event, the osteomyelitis may extend into the joint. The pattern of the inflammation produced by the organism recapitulates that seen in other locations. The cartilage is relatively resistant to destruction. Low-grade chronic synovitis leads to villous hypertrophy, which simulates rheumatoid arthritis.

Mice are subject at times to epizootic polyarthritis caused by *Streptobacillus moniliformis* (*4*, *5*). In this condition, the arthritis is a manifestation of a bacteremia. Conjunctivitis occurs early. The character of the articular lesions varies from fibrinous and necrotizing to proliferative. Organisms appear as pleomorphic bacilli and filaments. They

may be extracellular and intracellular. The most common site of involvement is the hind paw. While the infection is often fatal, there is considerable variation in susceptibility of various strains of mice to the organism. Surviving animals are left with ankylosis.

A similar problem exists with pleuropneumonia-like organisms (PPLO). These organisms occur with great frequency in normal mice, chiefly in the conjunctiva and nasal mucosa. Despite this fact, examination of thousands of mice has failed to reveal spontaneous arthritis due to PPLO (*6*). Some of these organisms are related to *S. moniliformis* and may be variants. When inoculated into mice, certain types produce polyarthritis which does not occur spontaneously. As will be seen directly, PPLO of certain types do cause spontaneous joint disease in rats.

An epizootic polyarthritis associated with pneumonia has been described in young white mice (*7*). The causative agent was filtrable, but PPLO could not be recovered by appropriate methods of culture. This fact suggested that the agent was viral.

Arthritis of mice is caused also by *Corynebacterium kutscheri* (*8*, *9*). It is particularly likely to occur in debilitated animals, such as those with tumors. The tibiotarsal region is the site of predilection. Microscopically, the lesion is characterized by coagulation necrosis and accumulation of masses of bacteria. The organism is a gram-positive diphtheroid. It is not to be confused with the *Pasteurella pseudotuberculosis rodentium*, a gram-negative bacillus that is responsible chiefly for the pseudotuberculosis of guinea pigs and rabbits. Pasteurella rarely causes arthritis in mice.

Epidemic polyarthritis, originally described in wild rats, occurs in laboratory rats as well (*10*). The tibiotarsal and radiocarpal joints are commonly involved, but at times small and other large peripheral joints and the vertebrae are also involved. The lesion is a suppurative one, affecting the soft tissues in and around the joints. It may regress in the course of several weeks or go on to ankylosis and deformity. The disease is due to a strain or strains of PPLO. Sporadic instances are infrequent.

Although rabbits are highly susceptible to experimental arthritis, they are not usually affected spontaneously. Arthritis may be a manifestation of staphylococcal infection of the navel in the newborn; or, at times, of sepsis of skin wounds in older animals. Sporadic infection with various organisms, including those of avian tuberculosis, has been recorded.

A different type of arthritis results from infection extending through abrasions of the soft plantar tissues. In older rats housed in hanging, metal, mesh cages, plantar decubitus ulcers develop frequently. These may become infected and give rise to suppurative arthritis (fig. 4) as well as cellulitis. Animals housed in cages with flat bottoms that are covered with sawdust are less prone to develop decubitis ulcers. However, if the sawdust is wet for excessive periods of time, the skin may become macerated and infected. Cellulitis ensues that may be confused with arthritis. Foreign-body reaction to the wood shavings may be seen. In two instances, we have observed associated actinomycosis (figs. 5 and 6).

Miscellaneous Articular Disease

1) *Fractures* occur with surprising frequency in mice, rats, and rabbits. In mice and rats, the fractures are usually in the tibia and fibula. In the region of the ankle, they may simulate acute or chronic arthritis (fig. 7). In rabbits, the vertebral column is affected more commonly but at times large peripheral joints may be involved.

2) *Congenital anomalies* in mice, not to be confused with arthritis, include bent tail and allied deformities. An anomaly apparently previously undescribed is failure of the secondary center of the femur to appear in mice and rats (fig. 8). This infantile cartilage persists into old age without giving rise to degenerative joint disease.

3) *Necrosis of the epiphysis or metaphysis* of the knees of mice is fairly common (fig. 9). The marrow and trabeculae are involved. The necrosis is of coagulative type and bacteria are not seen. The lesions may heal with fibrosis or occasionally by bony sclerosis that superficially resembles a medullary bone tumor (figs. 10 and 11). The process is limited sharply by the articular or epiphyseal plate, so that ordinarily the joint is not embarrassed. In rare instances, the epiphysis collapses or fracture of the bone, particularly the neck of the femur, takes place. Secondary osteoarthritis is the consequence (*11*).

4) *Chronic idiopathic synovitis and tenosynovitis*, with or without inflammation of adjacent tissues, such as the periosteum and subcutaneous tissue, occur infrequently in the knees, tarsal, and carpal regions of rats. Seropurulent fluid accumulates in the synovial spaces, while the tissues proper are infiltrated with chronic inflammatory cells and proliferating fibrous tissue. The overlying skin is intact and there is no suggestion of penetrating infection. Organisms are not seen in the exudate. In one such instance, we have recovered *S. moniliformis* by culture. In view of the frequency with which the organisms can be recovered from the blood of these animals and the failure to see them regularly in the exudate, conclusions as to their role in causing the lesion must be uncertain.

5) *Leukemic infiltration* of the synovial membrane commonly occurs in the knees of affected mice. The lesion is similar to that observed in a number of human patients with leukemia whose disease may be heralded by articular manifestations.

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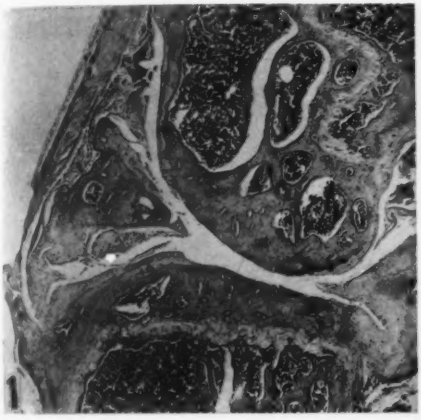
PLATE 92

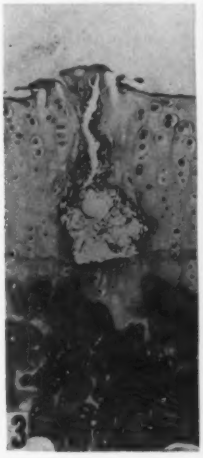
FIGURE 1.—Osteoarthritis of mouse knee. Articular cartilage of the weight-bearing portions of the condyles has been destroyed. Articulating surfaces are now the eburnated, exposed subchondral bone. × 22

FIGURE 2.—Chondromucoid degeneration of articular cartilage of rat knee. The cystic lesion in the deep portion of the cartilage is filled with metachromatic material. (*From* Sokoloff and Jay, A. M. A. Arch. Path., August, 1956.) Azure-eosin. × 115

FIGURE 3.—Cystic degeneration of articular cartilage of rat knee. No mucoid material persists in the cyst. The cyst communicates with the articular cavity through a fissure. (*From* Sokoloff and Jay, A. M. A. Arch. Path., August, 1956.) Hematoxylin and eosin. × 115

FIGURE 4.—Roentgenogram of suppurative osteoarthritis secondary to infection of plantar decubitus ulcer of rat. Extensive destruction of tarsal bones and the base of metatarsals is seen. The clouded appearance of the shafts of the metatarsal bones is caused by the associated new bone formation.





Sokoloff 971

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PLATE 93

FIGURE 5.—Cellulitis of forepaws of rat. The digits are swollen. The overlying skin is glistening, tense, and congested. Small crusts are at the tips of several of them.

FIGURE 6.—Photomicrograph of skin and subcutaneous tissue described in figure 5. An actinomycotic granule lies in the center of an abscess. In the framed area is a foreign body of sawdust. × 395

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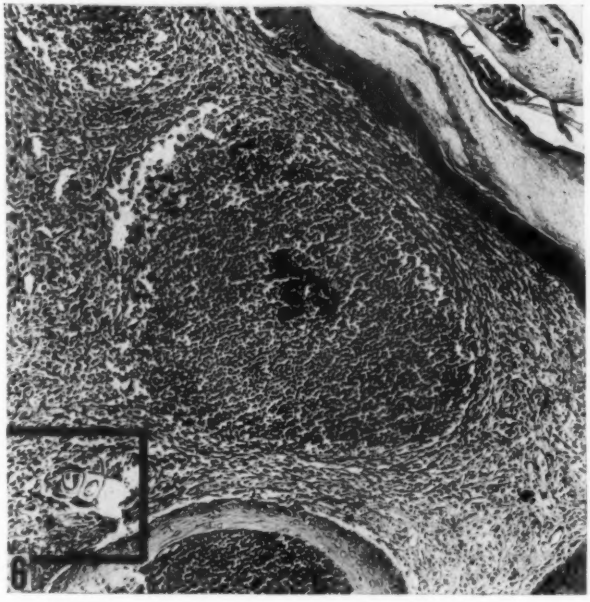
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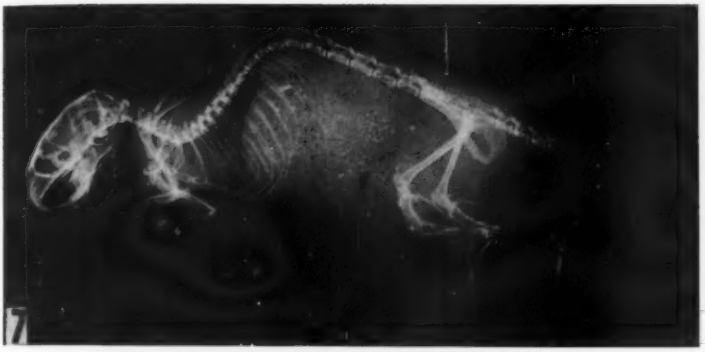
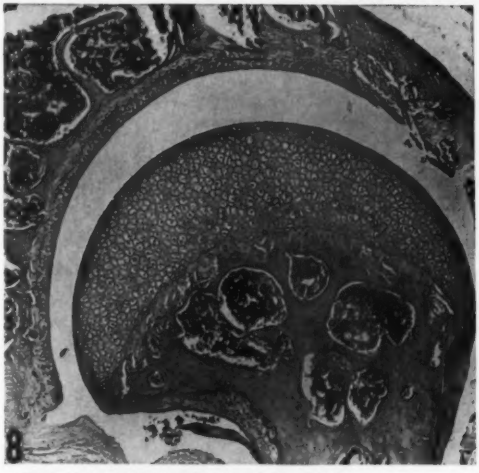
PLATE 94

FIGURE 7.—Roentgenogram of mouse that clinically presented a picture of acute arthritis of the left hind paw. There is a comminuted fracture of the tarsal and base of metatarsal bones, as well as the right tibia and fibula.

FIGURE 8.—Hip of mouse, 13 months old. There is no center of ossification in the epiphysis of the head of the femur. The acetabulum is ossified. (*From* Sokoloff and Jay, A. M. A. Arch. Path., August, 1956.) × 46

FIGURE 9.—Knee of mouse, 12 months old. The proximal epiphysis of the tibia is undergoing coagulation necrosis. The pallor of the marrow in this photograph reflects the loss of stainable nuclear material. (*Cf.* with marrow of the femur and the metaphysis of the tibia.) × 22





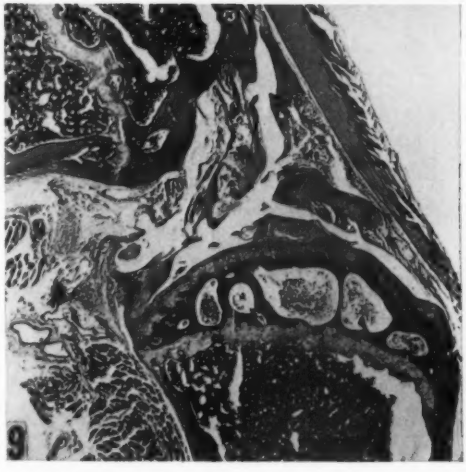
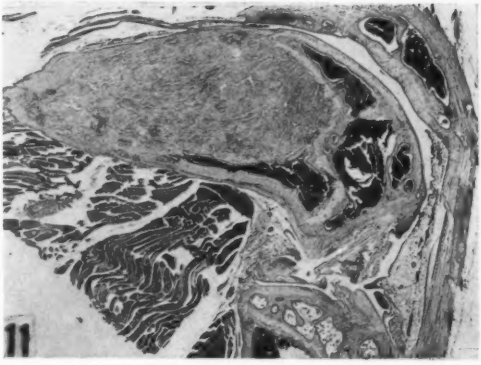


PLATE 95

FIGURE 10.—Roentgenogram of knees of mouse revealing unilateral, segmental opacity of metaphysis of the femur at *left*.

FIGURE 11.—Photomicrograph of figure 10. The opacity was caused by formation of membranous type of bone in the marrow space. It is presumed to be a late sequella of the type of necrosis illustrated in figure 9. (*From* Sokoloff and Habermann, A. M. A. Arch. Path., March, 1958.) × 18

10


11

The Identification and Control of Helminths in Laboratory Animals [1]

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Research workers in animal colonies all over the United States are becoming more cognizant of the importance of helminth-free laboratory animals. They recognize the fact that laboratory animals heavily infested with gastrointestinal parasites produce inferior biological products, make poor experimental or test animals, and greatly increase the cost of research. These workers are aware that heavily parasitized animals should not be used in research, especially in nutrition or blood studies, because normal physiologic reactions and blood counts are not obtained when such animals are used.

The pathologic changes produced in healthy laboratory animals by a few roundworms or tapeworms may be nil, but large numbers of helminths undoubtedly cause serious and often permanent injury. Under normal conditions a few gastrointestinal parasites in laboratory animals may produce no discernible ill effects, yet under experimental conditions the lowered resistance of the animals caused by equally few parasites may give rise to unreliable or misleading results.

In the identification of helminths in the laboratory animals—mice, rats, guinea pigs, hamsters, rabbits, monkeys, dogs, and cats—the salient points about parasitism in these animals will be presented, *e.g.*, factors that interfere with the diagnosis of parasitism, laboratory techniques for the diagnosis of parasitism, and characteristics of each of the parasites, so that any one can be recognized at necropsy or in a salt-flotation egg examination. Brief mention will also be made of the incidence, pathologic effects, diagnosis, and control of each of the helminths.

The incidences of the different parasites reported here were determined by salt-flotation fecal examinations and by injuries produced on the hosts in histopathologic studies on 6,209 animals that were obtained for necropsies from outside purchases and from the National Institutes of Health animal colonies from October 1, 1948 through February 1, 1952. Of the 6,209 animals, there were 2,029 mice, 1,266 rats, 2,277 guinea pigs, 440 rabbits, 117 monkeys, 25 cats, and 55 dogs.

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[1] Presented at the Armed Forces Institute of Pathology, Postgraduate Course, Washington, D. C., 1954.

[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

Diagnosis of Parasitic Infestations in Laboratory Animals

Parasitism is often difficult to recognize because of the insidious injury produced by parasites. Then, too, parasitic diseases are often accompanied by chronic diseases or malnutrition. When animals are thin, emaciated, and have rough hair coats, parasitism may be suspected and the diagnosis should be confirmed by fecal examinations or, when feasible, by necropsy of 1 or 2 sick animals.

Several methods of preparing fecal specimens for the diagnosis of parasitism have been used but the various techniques will not be given. However, it should be remembered that some parasite eggs are too heavy to be floated to the surface after centrifugation and can be found in the sediment of the centrifuge tube. When this is suspected, a small sample of the sediment should be placed on a glass slide, covered with a coverslip, and examined microscopically for parasite eggs.

Factors Interfering With Diagnosis

Negative fecal examinations do not always mean that the animals are free of helminths. The animals may have recently been exposed to a large number of infective larvae that have not reached maturity, so that there are no eggs in the fecal samples. In young and recently acquired animals that have been exposed to infection, it is advisable to make several fecal examinations at weekly or biweekly intervals.

If an animal has diarrhea, it is often difficult to determine the seriousness of parasitic infestation because of the diluted fecal specimen. Only a few parasite eggs may be observed, but the animal may be heavily infested with gastrointestinal parasites.

Tapeworm eggs are usually found in the feces in segments and, unless a segment of tapeworm is obtained with the fecal sample, the fecal examination may be negative. It is, therefore, imperative that the attendants of the animals mention the fact that segments of tapeworms have been observed in the animal's feces.

Composite Sample Technique

This technique is used to determine the kinds of intestinal parasites in cages of small laboratory animals rather than to determine the infestation in each animal in a cage. It is particularly useful in the diagnosis of intestinal parasitism in mice, rats, hamsters, and guinea pigs.

The cages of animals to be sampled are cleaned in the morning, and in the afternoon fresh fecal samples are collected from the sawdust pans in each cage. All samples from a cage are placed in a single centrifuge tube and the tube is numbered to correspond with the cage from which the sample is collected. When there are more than 3 rats or guinea pigs to a cage, the fecal pellets are broken in two and only half is used for the sample. The tubes containing the samples are placed in the refrigerator until time for examination. When they are removed, a small quantity of water is added to each and the fecal material is thoroughly macerated.

The samples are prepared for examination by the salt-flotation centrifugation method. After microscopic examination of the composite samples, the animals in cages in which parasites have been demonstrated can be isolated or given anthelmintic treatment.

Tapeworms of the Mouse and Rat

Hymenolepis nana

This adult tapeworm measures 10 to 45 mm. in length and is approximately 1 mm. in width. The scolex has 4 suckers and a rostellum armed with a row of hooks. The eggs are oval (fig. 1A) and within each egg are 2 membranes. The inner membrane gives rise to filiform projections at each pole. The eggs measure 40 to 60 μ in diameter and the oncosphere contains 3 pairs of lancet-like hooklets.

The life cycle of *H. nana* may be direct or indirect and a number of different species of insects, including fleas and beetles, may act as the intermediate host. The infective larval forms develop to mature worms in 15 to 20 days. Incidences as high as 5 percent in albino rats and 1 percent in albino mice have been recorded. Heavy infestations of *H. nana* sometimes cause catarrhal enteritis.

Diagnosis depends upon the microscopic identification of the characteristic eggs in the fecal examination or identification of the mature cestode.

Control.—Man is susceptible to infestation with this tapeworm, therefore strict precautionary measures are essential. Since this parasite has a direct life cycle, there is particular danger in carrying the infection by means of contaminated hands to uninfected animals. Cages should be cleaned and sterilized regularly, and every effort made to prevent contamination of the animal's feed and water. Infested animals should be dusted every month and feedbins must be protected from meal beetles. An insecticide consisting of 2 percent methoxychlor, 0.1 percent γ-benzene hexachloride (Lindane®), and 97.9 percent talc, used as a dusting powder, is very effective in controlling the external parasites of mice and rats. Group treatment for this tapeworm consists of 50 mg. of lead arsenate in 10 gm. of ground mouse feed and 100 mg. of lead arsenate to 20 gm. of ground rat feed. We have found this medicated feed to be effective in the removal of this tapeworm.

Hymenolepis diminuta

The other tapeworm found in mice and rats is *Hymenolepis diminuta*. The adult of this species measures from 10 to 60 mm. in length and is 4 mm. in width. The scolex has 4 suckers and a small unarmed rostellum. The eggs are almost spherical (fig. 1B), yellowish in color, and from 54 to 86 μ in diameter. The oncosphere contains 3 pairs of hooklets in a fan-shaped arrangement.

The life cycle of *H. diminuta* requires an intermediate host. Beetles, fleas, and grain-eating arthropods serve as intermediate hosts. The ingested cysticercoid develops to the adult tapeworm in the mouse and rat

in from 18 to 20 days. Incidences as high as 2.5 percent in mice and 15 percent in rats have been recorded. Heavy infestations of this tapeworm may cause catarrhal enteritis.

Diagnosis depends upon the microscopic identification of the characteristic eggs in fecal examinations or identification of the mature cestode.

Control.—Animals should be dusted regularly, and meal beetles and other grain-eating insects should be prevented from getting into the feed. Lead arsenate in the feed is also effective for the removal of *H. diminuta*.

Roundworms of the Mouse and Rat

Heterakis spumosa

This nematode is rarely found in the laboratory rat. The males are from 6 to 10 mm. long and are 260 μ wide; the females, from 7 to 13 mm. long and 740 μ wide. The ascarid-like eggs (fig. 2A) have thick, mammillated shells and measure from 55 to 60 μ in diameter.

The life cycle is direct. The eggs in the feces become infective in 27 days and after ingestion develop to maturity in the rat's cecum and colon in 26 to 47 days. Winfield (*1*) reported that no pathologic changes were grossly visible in experimentally infected laboratory mice or rats.

Diagnosis depends upon the microscopic identification of the characteristic eggs or upon identification of the mature nematode.

Control.—Cages should be cleaned regularly and contamination of the animals' feed and water prevented. One gm. of phenothiazine mixed with 10 cc. of molasses added to 20 gm. of ground rat feed is an effective treatment.

Syphacia obvelata

This oxyurid (fig. 3B) is a common parasite of the rat and also occurs in the mouse. Males are 1.3 mm. long and 110 μ wide; females are 3.5 to 5.7 mm. long and 100 to 250 μ wide. The eggs have one straight side (fig. 2B) and are kidney-shaped and thin-shelled. They measure from 110 to 142 μ in length and 30 to 40 μ in breadth.

In the life cycle the eggs undergo a short period of development to reach the infective stage, and the infective larvae reach maturity in the cecum and colon in from 15 to 28 days. *S. obvelata* has been found in 14 percent of the rats and 2 percent of the mice. No lesions attributable to this parasite have been observed.

Diagnosis depends upon the identification of the characteristic eggs and worms in the fecal examinations.

Control.—Cages should be cleaned regularly and fecal contamination of feed and water prevented. Group treatment of 10 infested mice with 400 mg. of piperazine adipate in 100 cc. of water and 250 mg. of the drug in 50 cc. of water per rat is effective (*2*).

Aspicularis tetraptera

This oxyurid nematode (fig. 3A) of the mouse measures approximately 2 mm. in length and 200 μ in width (fig. 2C). The eggs are symmetrical,

thin-shelled, and measure from 84 to 90 μ in length and 34 to 40 μ in breadth.

The life cycle is direct and the mature worms are found in the cecum and colon in 15 to 30 days after ingestion of the infective eggs. *A. tetraptera* has been found in 4.5 percent of the mice examined. No lesions have been attributed to these worms.

Diagnosis depends upon the identification of the characteristic eggs in the fecal examinations.

Control.—Group treatment of infected animals is the same as for *S. obvelata.*

Trichosomoides crassicauda

This nematode is occasionally found in the urinary tract of the laboratory rat. The female measures 10 mm. in length and 200 μ in diameter. The male, which is from 1.5 to 3.5 mm. in length, is found in the genital tract of the female nematode. The eggs, which are thick-shelled (fig. 4) and brown, with polar plugs, measure 60 by 30 μ. They are passed in the urine. The infective eggs hatch in the stomach, pass to the lungs and body cavities within a few hours, and become embedded in the mucosa of the bladder in 3 to 6 weeks (fig. 5). Segments are also seen in the renal pelvis (fig. 6).

Other Nematodes of the Mouse and Rat

Nippostrongylus muris and *Capillaria hepatica* have not been observed in the laboratory rat or mouse. Both of these species are frequently found in wild rats.

Roundworms of the Guinea Pigs

Paraspidodera uncinata

The males of this roundworm are 11 mm. in length and 300 μ in width; the females, 16 mm. by 400 μ. The eggs are of ascarid-type and similar to those of *Heterakis spumosa.* They are ellipsoidal and measure 43 by 31 μ.

The life cycle of *P. uncinata* is direct and the mature worms are found in the cecum and colon. The parasite develops to maturity in about 2 months. No lesions attributable to this parasite have been observed. This roundworm has not been found in NIH guinea pigs, but incidences as high as 8.5 percent have been reported in guinea pigs kept in outside pens.

Diagnosis depends upon the identification of the adult nematode or of the characteristic eggs in fecal specimens.

Control.—Contamination of the feed and water should be prevented and the cages cleaned regularly. Group treatment of infected animals with 1 gm. of phenothiazine per 20 gm. of molasses guinea-pig feed may be effective.

Roundworms of the Rabbit

Passalurus ambiguus

This oxyurid of the rabbit is found in the cecum and colon. The males are 4.0 mm. in length and 300 μ in width; the females 5.5 mm. in length and 500 μ in width. The eggs are slightly flat on one side and measure 103 by 43 μ (fig. 7).

This pinworm has a direct life cycle. No pathologic changes have been attributed to it.

Diagnosis is made by finding the characteristic eggs in the feces.

Control.—Measures include prevention of contamination of feed and water by infective eggs and regular cleaning of the cages. One gm. of phenothiazine mixed in 50 gm. of molasses rabbit feed may be an effective treatment.

Roundworms of the Rhesus Monkey

Oesophagostomum apiostomum

The adult females of this strongylid nematode measure 8.5 to 10.5 mm. in length and 0.2 to 0.3 mm. in width, and the males are 8 to 10 mm. in length and 0.3 to 0.35 mm. in width. The dimensions of the eggs are 60 to 63 μ by 27 to 40 μ (fig. 8A).

The mature larvae invade the wall of the cecum where they stimulate nodule formation. After a period of development, they break out of the nodules and migrate to the lumen where they develop into adult worms.

Heavy infestations may cause diarrhea. Microscopically, nodules 1 to 2 mm. in diameter are seen in the muscular wall. They have caseous centers that may become calcified and are enclosed in fibrous capsules that are infiltrated with eosinophils, lymphocytes, and monocytes. Several foreign-body giant cells may be seen around the periphery. Occasionally, an ulcer is seen in the mucosa with an irregular tract leading to a nodule in the muscularis. The tract shows infiltration of eosinophils, lymphocytes, and neutrophils. Thirty percent of the rhesus monkeys examined have been infested with this parasite.

Diagnosis depends upon the identification of the characteristic eggs in the fecal examinations or upon identification of the mature nematode.

Control.—Nematode eggs passed in the feces from infected animals develop into infective larvae in 1 to 2 days and sanitary measures should be taken to prevent contamination of the animals' feed and water with infective larvae. The administration of 1 gm. of phenothiazine in the feed or in a capsule is an effective treatment.

Strongyloides intestinalis

This nematode is found in the small intestines. Apparently only the females are found in the definitive host cycle; they are 1 mm. in length and 30 to 75 μ in width. The eggs are embryonated, oval, and measure from 50 to 57 μ long and 30 to 35 μ wide (fig. 8B).

The infective larvae may gain entrance into the body by contaminated feed or water or by penetrating the skin. They enter the general circulation, break out of the alveoli of the lungs, pass up to the trachea, are swallowed and reach the small intestines where they develop into mature worms. Heavy infestations may cause catarrhal enteritis and diarrhea. Fifty percent of the monkeys examined are infested with this parasite.

Diagnosis depends upon the identification of the embryonated eggs in the fecal examination or by identification of the mature worm.

Control.—Prevent contamination of the feed and water by infective larvae and remove feces from the cages daily.

Capillaria hepatica

The adult male capillarid is 4 mm. long and 100 μ in diameter. The females are 10 mm. long and are 200 μ in width. The eggs measure 56 to 43 μ. They are brown, pitted, thick-shelled, and have bipolar plugs. The masses of eggs in the tissues (fig. 9) are surrounded by dense strands of fibrous connective tissue, infiltrated with eosinophils, lymphocytes, and monocytes. The eggs become infective in from 4 to 6 weeks, and, when swallowed, the embryonated eggs hatch in the intestines, pass through the wall of the cecum, enter the bloodstream, and migrate to the liver in 5 to 10 days.

No treatment for this parasite is known.

Tapeworms in the Dog and Cat

Dipylidium caninum

This cestode may attain a length of from 100 to 700 mm. and the maximum width of the mature proglottids is 3.2 mm. The rostellum is armed with 30 to 150 thorn-shaped hooklets; the mature proglottids are much longer than they are wide, and are elliptical in shape. The eggs are enclosed within egg capsules (fig. 10A). They are thin-shelled, from 35 to 60 μ in diameter, and each egg contains a 6-hooked embryo.

D. caninum requires an intermediate host and passes part of its life cycle in the flea or louse. Infective eggs develop to the systicercoid stage in the intermediate host in 30 days, and after the infested flea or louse is ingested by the dog or cat the tapeworm reaches maturity in the small intestines of the definite host in 2 or 3 weeks. Heavy infestations cause colic and chronic enteritis.

Diagnosis depends upon the identification of double-pore segments of the tapeworm or of the egg-capsules in the feces or upon the presence of the eggs in fecal examinations.

Control.—Man is susceptible to this tapeworm and precautionary measures should be taken to prevent infestation after handling dogs and cats or their bedding. The animals and their bedding should be dusted frequently. Nemural®[3] (1 tablet per 8 lbs. of body weight) is an effective drug for the removal of *D. caninum* from mature dogs and cats.

[3] Drocarbil: arecoline salt of acetarsone. Arecoline: methyl 1,2,5,6,-tetrahydro-1-methyl nicotinate. Acetarsone (stovarsal) *N*-acetyl-4-hydroxy-*m*-arsanatic acid, or 3-acetamide-4-hydroxybenzenearsonic acid.

Taenia pisiformis

This cestode is 500 mm. in length. The gravid segments are about 5 mm. in width and longer than they are wide. The rostellum is armed with a double row of 34 to 48 hooklets. The eggs measure 36 to 40 μ long and 31 to 36 μ wide, and each egg contains an embryo with 3 pairs of hooklets.

T. pisiformis passes a part of its life cycle in rabbits, squirrels, rats, and other rodents. The cysticerci are found in the peritoneal cavity (fig. 11), attached to the viscera of infested rodents, about 22 days after ingestion of infective eggs. The cysticerci are 10 mm. in diameter. The dog and, occasionally, the cat become infected when they eat the parasitized rodent. Heavy infestations cause colic and chronic enteritis.

Diagnosis depends upon the identification of the tapeworm segments in the feces or upon the presence of the eggs in the fecal examination.

Control.—Prevent dogs and cats from consuming infested rodent carcasses and treat infested dogs and cats. Nemural is an effective drug for the removal of *T. pisiformis* from dogs and cats.

Taenia taeniaformis

This species is the common tapeworm of the cat but is also found in dogs and other carnivores. Its length varies from 15 to 60 cm. The gravid segments are bell-shaped and 5 or 6 mm. wide. The rostellum is armed with a double row of 26 to 62 hooklets. The eggs are thick-shelled, globular, and 31 to 37 μ in diameter (fig. 10B).

The intermediate hosts of this parasite are rodents, especially rats, mice, and rabbits. Ivory-white cysts *Cysticercus fasciolaris* (fig. 11B), develop on the liver after the intermediate host ingests infective eggs from cats harboring *T. taeniaformis*. These liver cysts contain a segmented worm and when the rodent is eaten by a cat, the encysted worm, *strobilocercus*, develops into an adult tapeworm in the small intestines of the cat. Heavy infestation causes colic and chronic enteritis.

Diagnosis: In the intermediate hosts the ivory cysts of *C. fasciolaris* in the liver (fig. 12) are characteristic, and in the cat the tapeworm is diagnosed by the identification of the mature worm or by the segments in the feces. It should be mentioned that the identification of the particular species of *Taenia* cannot be made from the characteristics of the ova alone. However, the treatment of choice for tapeworms in mature dogs and cats is the same (Nemural), and from a practical point of view the identity of the tapeworm makes little difference.

Control.—Keep cats out of animal colonies; prevent stray mice and rats from gaining access to infected cat feces and treat infected cats.

Roundworms in the Dog and Cat

Ancylostoma caninum

This is the common hookworm of dogs and cats. The length of the male varies from 9 to 12 mm. and that of the females from 15 to 20 mm.

The eggs are ellipsoidal with thin walls and measure 50 to 60 μ in length and 34 to 40 μ in breadth (fig. 13A).

The life cycle of this nematode is direct and infected larvae may produce infection by three routes: 1) ingestion, 2) penetration of the skin, and 3) prenatal infection. The common method of infection in the dog is by ingestion of contaminated feed. By this route the infective larvae develop in the gastric glands of the stomach and in the glands of Lieberkühn of the small intestines, and then reach maturity in the lumen of the small intestines. The second method of infection is by penetration of the skin. After penetration, the larvae enter the bloodstream, then the lungs, pass up the trachea, and reach the small intestines where they mature. Approximately 5 weeks are required to complete development from the infective larvae to the adult stage. In dogs, Herrick (*3*) has shown that *A. caninum* larvae complete their life cycle in 15 to 26 days; in cats 22 to 25 days are required. In prenatal infection, eggs may appear in the feces of puppies 10 to 12 days after birth.

The primary changes produced by hookworms are in the small intestines and in the blood. The worms attach themselves to the mucosa and draw blood and then migrate to a new area to repeat the process. This procedure causes numerous open lesions and bleeding areas in the mucosa. In heavy infestations, these worms produce anemia.

Diagnosis depends upon the identification of the mature worm and of the characteristic eggs in the fecal examinations.

Control.—Treat infected animals, clean cages regularly, and dispose of feces to prevent exposure to infective larvae. *N*-butyl chloride and toluene (0.1 cc. per lb. of body weight) are effective drugs for the removal of hookworms from dogs and cats. It should be remembered that cats are sensitive to chlorinated hydrocarbons and should be watched for evidence of toxicity. However, it has been our experience that mature cats withstand the treatment very well.

Trichuris vulpis

The whipworm is found in the cecum and colon of the dog and does not occur in cats in North America, Enzie (*4*). The length of the males and females varies from 45 to 75 mm. The eggs have thick shells, are barrel-shaped and brown, have plugs at each end, measure 70 to 80 μ in length and are 32 to 40 μ in width (fig. 13D).

The larvae penetrate the mucosa of the small intestines after the infective eggs are eaten by the dog, and after a period of development they return to the lumen and pass to the cecum and colon where they reach maturity in from 70 to 90 days. Heavy infestations cause colic and chronic enteritis, and the mucosa of the cecum shows areas of thickening and inflammation due to the attachments of the nematode.

Diagnosis depends upon the identification of the mature worms and of the characteristic eggs in the fecal examinations.

Control.—Treat infected animals, clean cages regularly, and protect the animals' feed and water from becoming contaminated with infective

whipworm eggs. *N*-butyl chloride is an effective anthelmintic for whipworms, but the efficacy of the treatment depends upon the drug entering into the cecum.

Capillaria felis-cati

This capillarid is found in the urinary bladder of the cat. The males measure 25.5 mm. in length and the females from 28 to 32 mm. The eggs are oval (fig. 14A) with squarely flattened opercula at each end, have a thick, spotted shell, and are 51 to 65 μ long and 24 to 32 μ wide. The life cycle of this parasite is unknown. This species is reported to be slightly pathogenic.

Diagnosis depends upon the identification of the characteristic eggs in urine and fecal examinations.

Control.—Clean cages regularly and prevent contamination of the animals' feed and water by infective eggs.

Capillaria aerophila

This capillarid is found in the trachea (figs. 15 and 16) and bronchi of dogs and cats. The males measure 15 to 25 mm. in length and the females 20 to 40 mm. The eggs are oval, deep brown, with large plugs at each end, and have a striated shell (fig. 14B). The eggs measure 58 to 70 μ in length and 29 to 40 μ in width.

The life cycle is direct. After the embryonated eggs are ingested, the larvae penetrate the mucosa of the small intestines, enter the bloodstream, and reach the lungs where they develop to maturity. The lung worms reach maturity 40 days after ingestion. Heavy infestation may cause bronchitis and bronchial pneumonia.

Diagnosis depends upon the identification of the characteristic eggs in material swabbed from the larynx and pharynx and in the fecal examinations.

Control.—Clean cages regularly and prevent the animals' food and water from becoming contaminated with infective lung-worm eggs.

Capillaria plica

This capillarid is found in the urinary bladder and renal pelvis of the cat and dog. The males measure 13 to 15 mm. in length and the females from 30 to 36 mm. The eggs are oval (fig. 14C) and have colorless, pitted shells with irregularly shaped plugs at each end. They measure approximately 65 by 30 μ.

The life cycle of this parasite is unknown. This species is reported to be slightly pathogenic.

Diagnosis depends upon the identification of the characteristic eggs in the urine and fecal examinations.

Control.—Clean cages regularly and prevent the animals' feed and water from becoming contaminated with infective eggs.

Dirofilaria immitis

This filarid worm is recovered primarily from the right ventricle and pulmonary artery of the dog, cat, fox, and wolf. The disease is found principally in the southern States but has been reported throughout the United States. The males are about 16 cm. long and the females about 25 cm., and both are about 1 mm. in width. The microfilariae vary in length from 218 to 329 μ and are from 5 to 6 μ wide (fig. 17).

The microfilariae live in the bloodstream and may persist for months or longer. The life cycle is continued by the bite of a bloodsucking arthropod, dog flea, cat flea, or mosquito. The microfilariae develop to the infective stage within 120 hours in the flea and within 240 hours in the mosquito. The life cycle is completed by the bite of the intermediate host and the passage of the infective larvae into the bloodstream of the dog and cat. Then the larvae develop to maturity in the tissues and migrate to the heart or pulmonary vessels. The primary lesions produced by *D. immitis* are chronic endocarditis, dilation of the right ventricle, congestion of the lungs, enlargement of the spleen, and ascites.

Diagnosis depends upon the demonstration of the microfilariae in the bloodstream.

Control.—Prevent dogs and cats, as much as possible, from being bitten by fleas and mosquitoes, and treat the infected animals. Sodium antimony (Fauding®), 1-diethylcarbamyl-4-methylpiperazine HCl (Carcide®) or diethylcarbamazine citrate (Hetrazan®), and sodium arsenamide are reportedly effective treatments for heartworms in dogs.

Aelurostrongylus abstrusus

This nematode is occasionally found in the lungs of cats. The males are 5 mm. in length and the females are twice as long. The eggs are subglobular and are 60 to 85 μ long and 50 to 80 μ wide.

The adult lungworms live in the pulmonary arteries. The eggs develop in the capillaries (figs. 20, 21, and 22); the larvae gain entrance to the alveoli (figs. 18 and 19) where they pass up the trachea to the oral cavity, are swallowed, and pass out of the body in the feces. Hobmaier and Hobmaier (*5*) have shown that snails and slugs are the necessary intermediate hosts. The larvae develop into infective larvae after 4 or 5 weeks in the mollusc. The transport hosts—frogs, toads, lizards, snakes, birds, and mammals—become infected by ingestion of the infected intermediate hosts. Infections of the cats occur through ingestion of either the infected intermediate or transport hosts. The infective larvae are liberated in the stomach of the cat and penetrate the stomach or upper part of the duodenum where they migrate to the lungs within 1 or 2 days. Five weeks later the larvae develop into mature worms.

The adult worms cause very little damage to the pulmonary arteries. The eggs in the capillaries may produce transient thrombi throughout the lungs and in heavy infections coughing, intestinal disturbances, and emaciation are prominent manifestations.

Control.—Prevent cats from consuming intermediate and transport hosts. No treatment for this parasite has been reported.

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(*3*) Herrick, C. A.: A quantitative study of infection with *Ancylostoma caninum* in dogs. Am. J. Hyg. 8: 125–157, 1928.

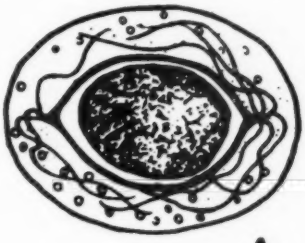
(*4*) Enzie, F. D.: Do whipworms occur in domestic cats in North America? J. Am. Vet. M. A. 119: 210–213, 1951.

(*5*) Hobmaier, M., and Hobmaier, A.: Mammalian phase of the lungworm *Aelurostrongylus abstrusus* in the cat. J. Am. Vet. M. A. 87: 191–198, 1935.

Plate 96

Figure 1.—A. *Hymenolepis nana.*
B. *Hymenolepis diminuta.*

Figure 2.—A. *Heterakis spumosa.* × 160
B. *Syphacia obvelata.* × 160
C. *Aspicularis tetraptera.* × 160



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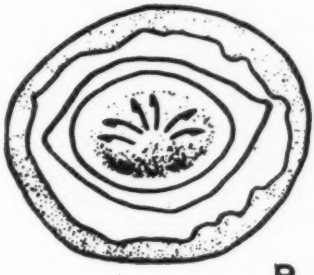

B.

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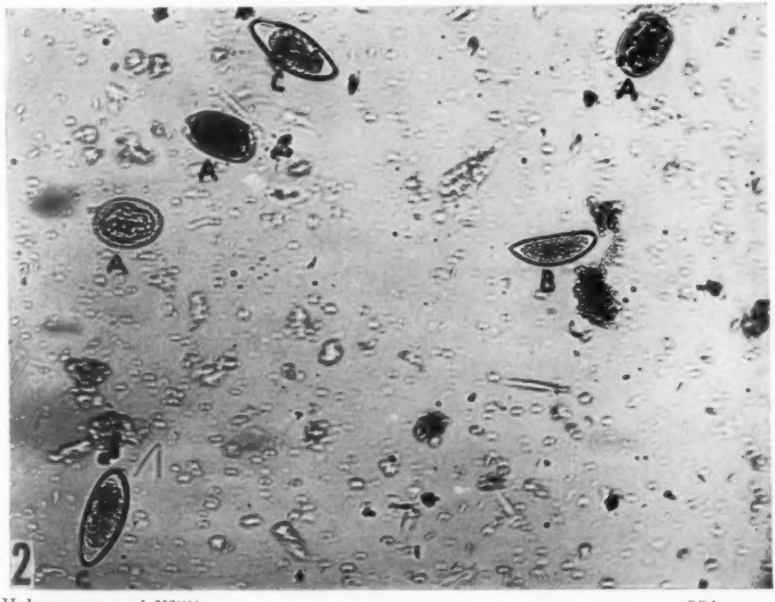
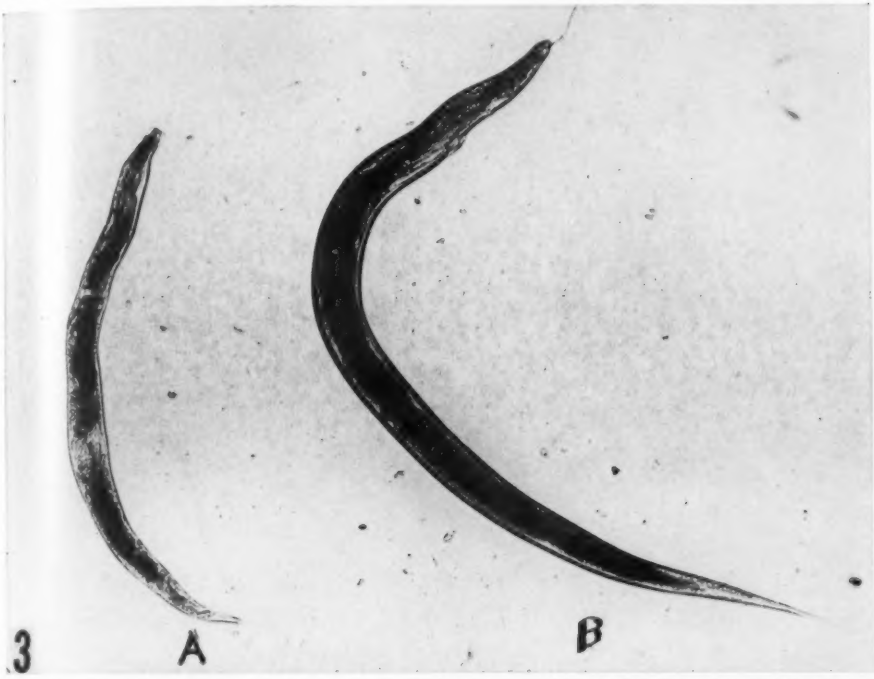


PLATE 97

FIGURE 3.—A. *Aspicularis tetraptera.* × 25
B. *Syphacia obvelata.* × 25

FIGURE 4.—*Trichosomoides crassicauda.* × 335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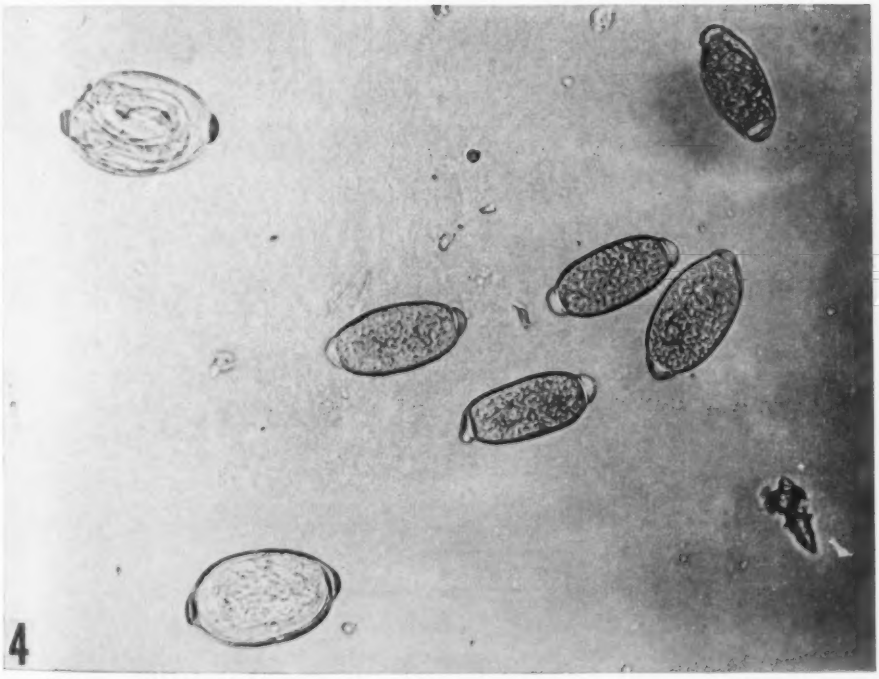
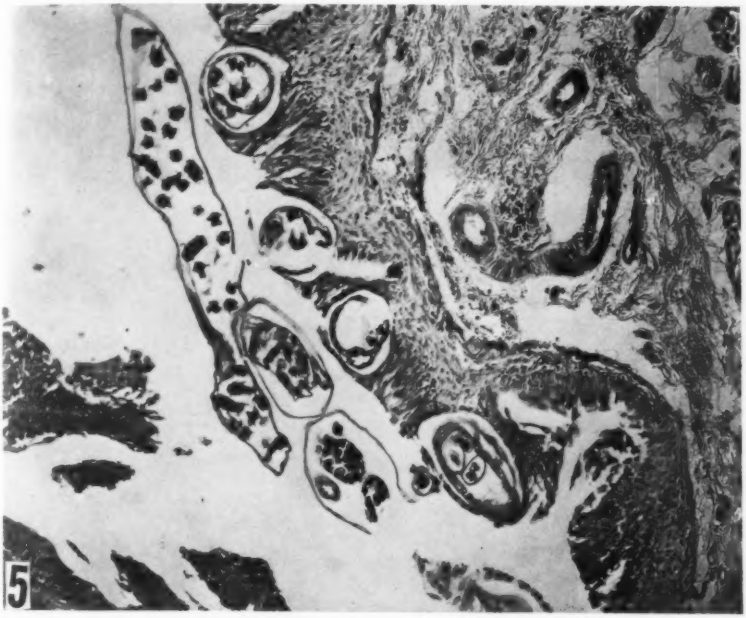

4

PLATE 98

FIGURE 5.—*Trichosomoides crassicauda.* Lumen and mucosal epithelium of bladder. × 90

FIGURE 6.—Segments of *Trichosomoides crassicauda* in renal pelvis in submucosa. × 145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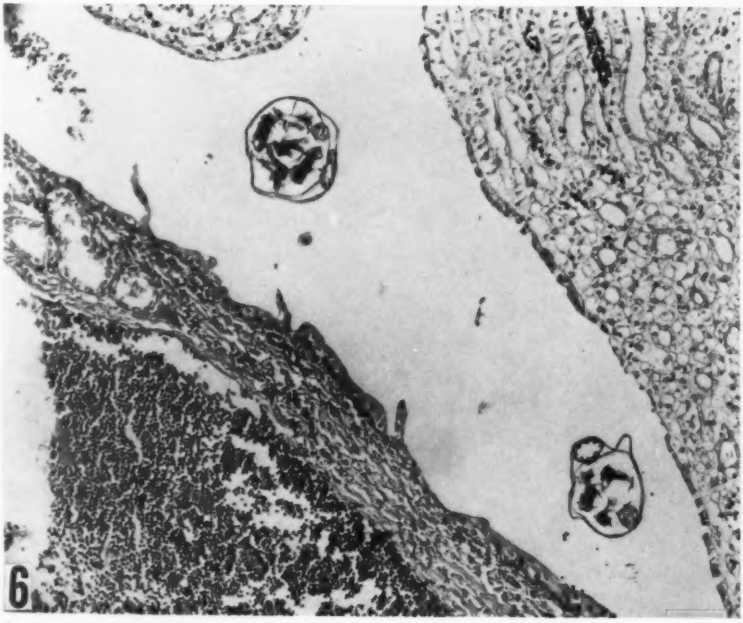
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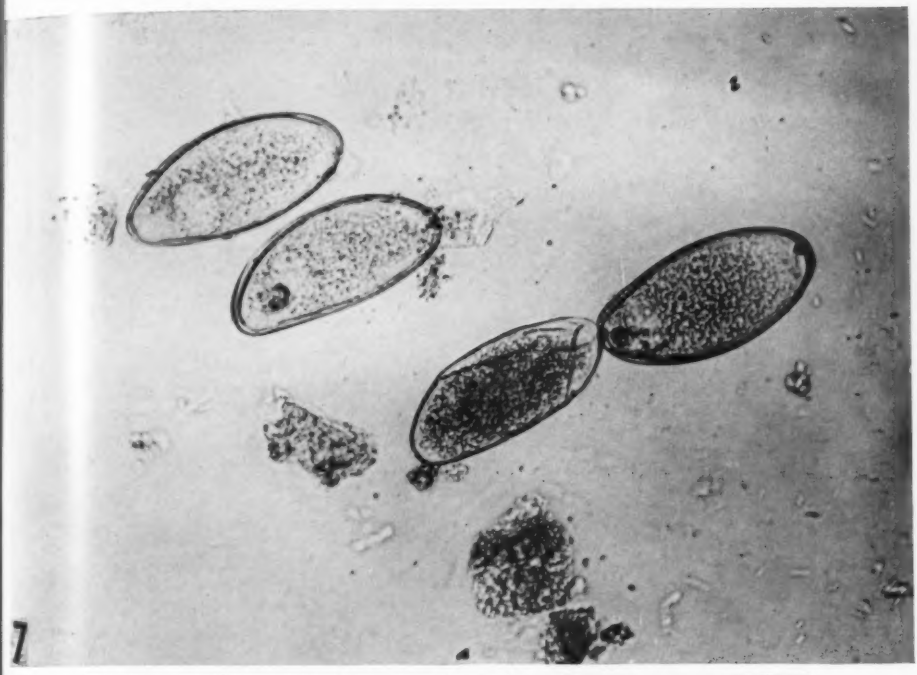
PLATE 99

FIGURE 7.—*Passalurus ambiguus* ova. × 335

FIGURE 8.—A. *Oesophagostomum apiostomum.* × 395
B. *Strongyloides intestinalis.* × 395



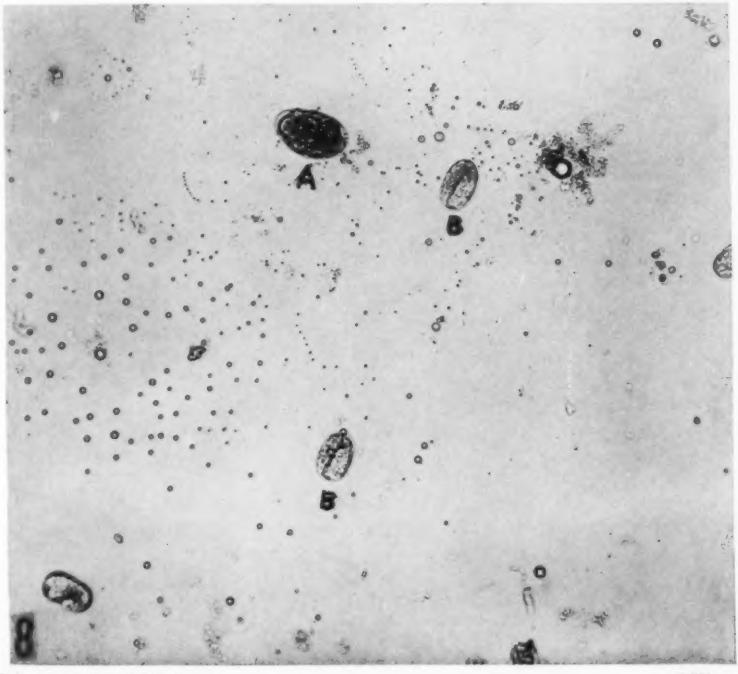


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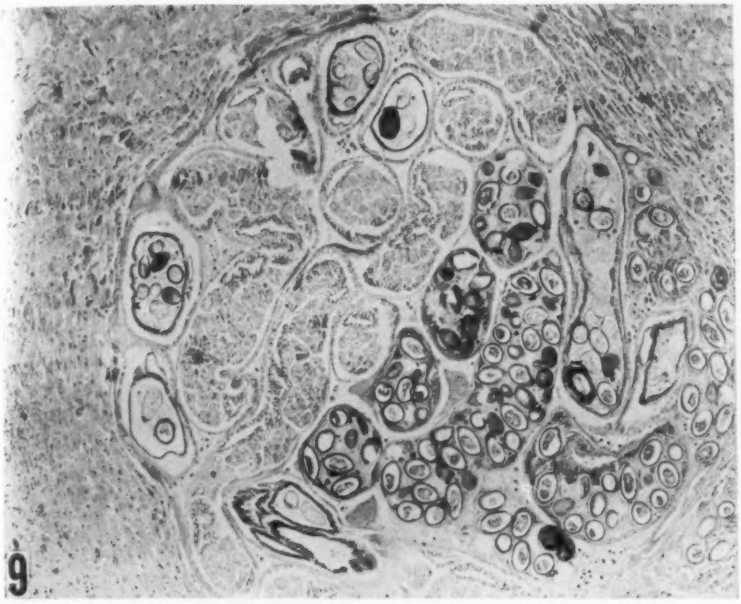
PLATE 100

FIGURE 9.—*Capillaria hepatica.* Liver of monkey. × 100

FIGURE 10.—A. *Dipylidium caninum.* × 160
B. *Taenia taeniaformis.* × 160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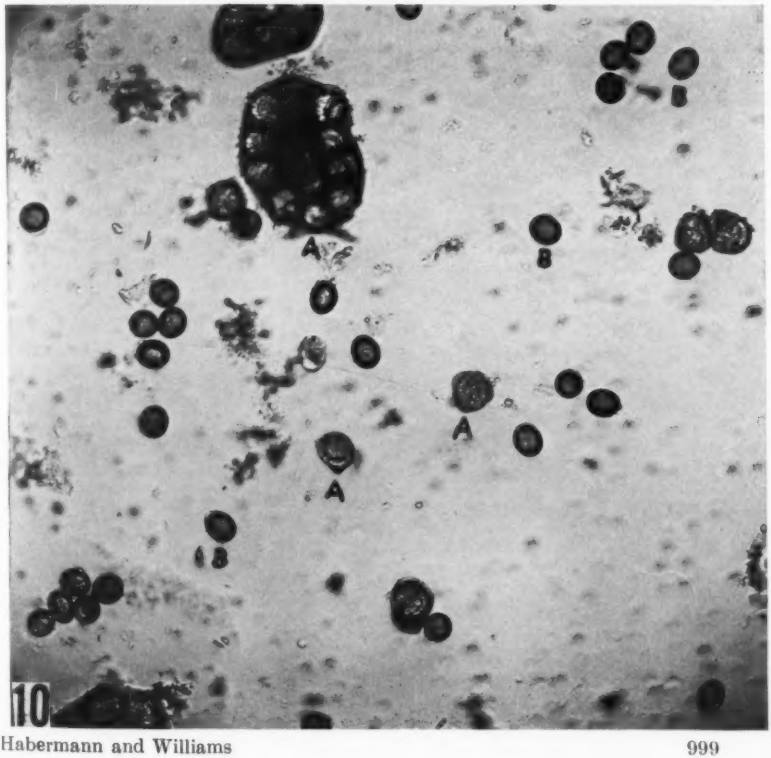
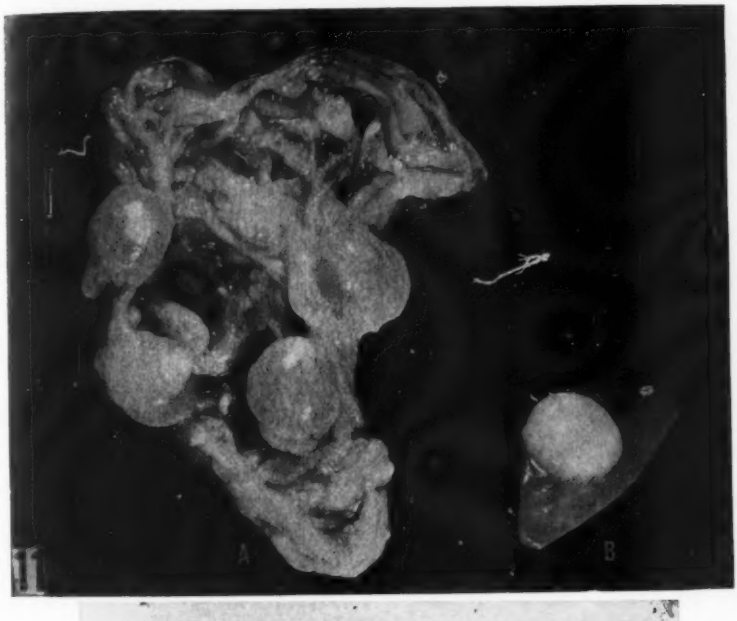

A
B
A
A
B
10

PLATE 101

FIGURE 11.—A. *Cysticercus pisiformis.* The larval stage of *Taenia pisiformis.*
B. *Cysticercus fasciolaris.* The larval stage of *Taenia taeniaformis.*

FIGURE 12.—*Cysticercus fasciolaris.* The larval stage of *Taenia taeniaformis* in the liver of a mouse. × 22

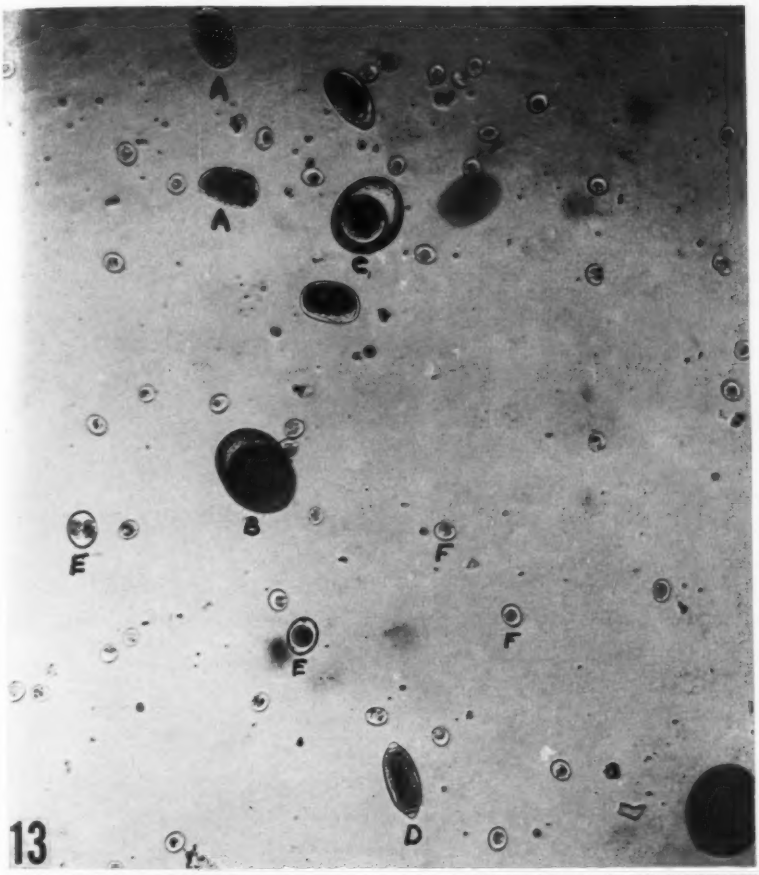

A
B

12

PLATE 102

FIGURE 13.—A. *Ancylostoma caninum.* × 135
B. *Toxocara canis.* × 135
C. *Toxascaris leonina.* × 135
D. *Trichuris vulpis.* × 135
E. *Isospora rivolta.* × 135
F. *Isospora bigemina.* × 135

FIGURE 14.—A. *Capillaria felis-cati.*
B. *Capillaria aerophila.*
C. *Capillaria plica.*
D. *Trichuris vulpis* (from Enzie).


A
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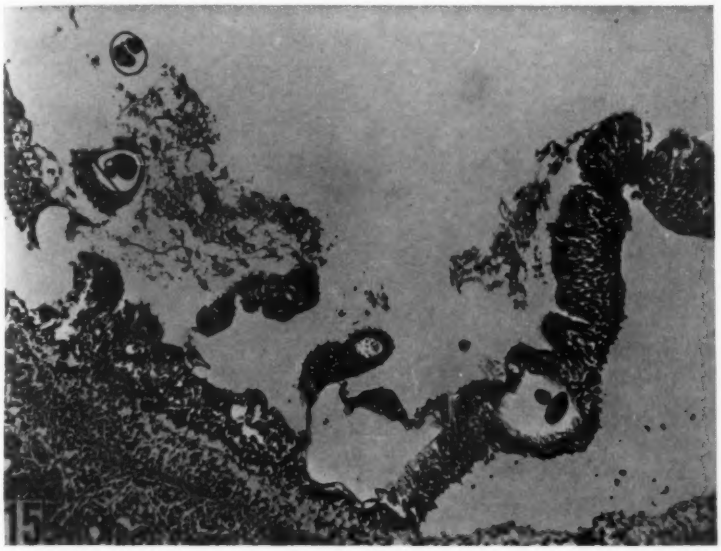
C
B
E
F
F
E
D
13

25 μ
A
B
C
D
14

PLATE 103

FIGURE 15.—*Capillaria aerophila.* Cross-section and ova from trachea of cat. × 60

FIGURE 16.—*Capillaria aerophila* ova. Trachea of cat. × 100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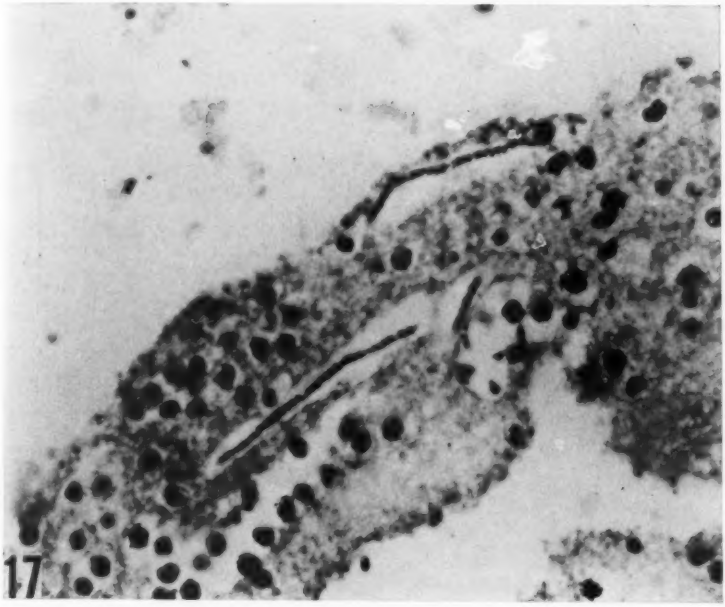
16

PLATE 104

FIGURE 17.—*Dirofilaria immitis.* Microfilaria in heart of dog. × 750

FIGURE 18.—*Capillaria* larvae and cross-section in lung of cat. × 60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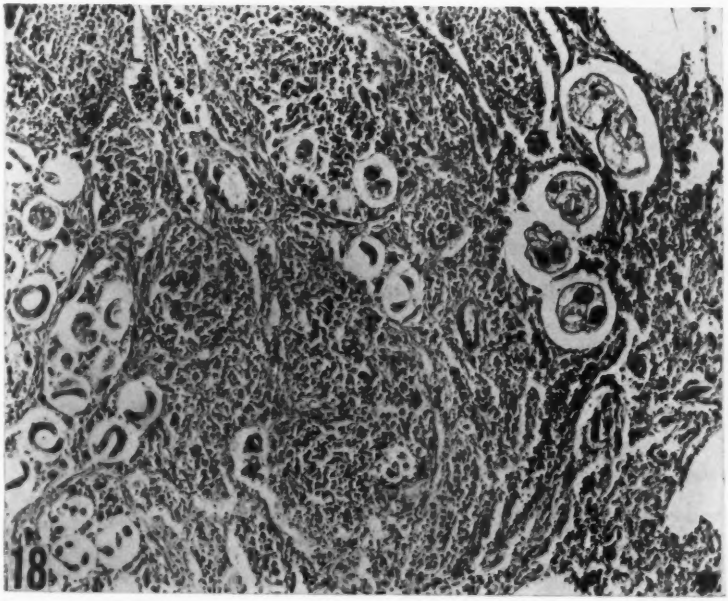

18

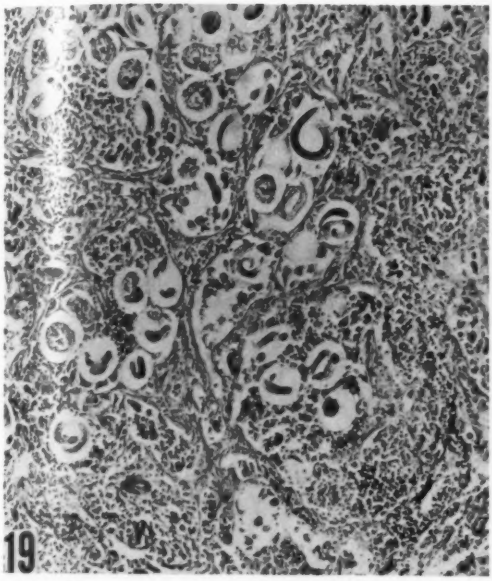
PLATE 105

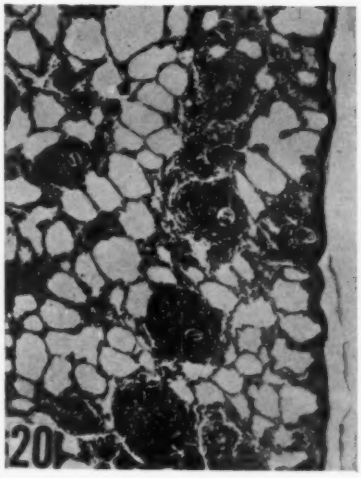
FIGURE 19.—*Capillaria* larvae in lung of cat. × 60

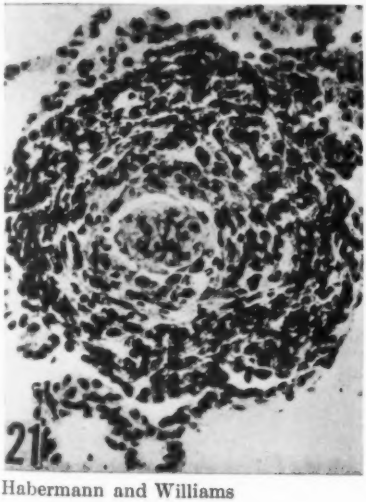
FIGURE 20.—*Aelurostrongylus abstrusus.* Lung of cat showing pseudotubercles containing eggs and embryos (from Cameron).

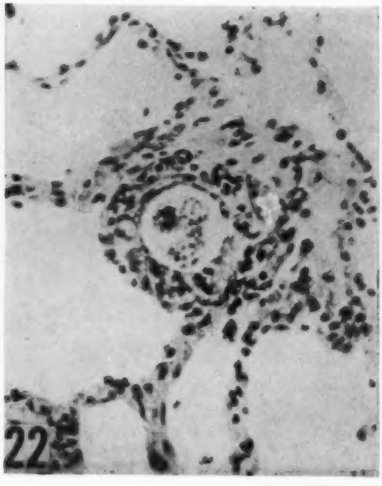
FIGURE 21.—*Aelurostrongylus abstrusus.* Lung of cat showing pseudotubercle containing developing egg almost occluding the small blood vessels (Cameron).

FIGURE 22.—*Aelurostrongylus abstrusus.* Lung of cat. An older egg in which the larva is formed and catarrhal exudate has disappeared.









Habermann and Williams

1009

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The Journal of the National Cancer Institute is published monthly by the National Cancer Institute of the National Institutes of Health, Bethesda, Md. Twelve issues are published each year in two volumes, beginning with number 1 in July and January. The Journal publishes original observations and investigations in laboratory and clinical research in cancer. Manuscripts are considered for publication with the understanding that they have not been published previously.

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CONTENTS

JOURNAL OF THE NATIONAL CANCER INSTITUTE • VOL 20 • NO. 5 • MAY 1958

Page

Epidemic diarrheal diseases of suckling mice. *Alwin M. Pappenheimer* 861

Lymphocytic choriomeningitis. *Fred D. Maurer* 867

Mouse poliomyelitis or Theiler's mouse encephalomyelitis. *Fred D. Maurer* .. 871

Mouse pox (infectious ectromelia). *Leon Z. Saunders* 875

Pathology of infection with the JHM virus. *Alwin M. Pappenheimer* 879

Tyzzer's disease. *Leon Z. Saunders* 893

Myositis in guinea pigs. *Leon Z. Saunders* 899

Salivary-gland virus disease of guinea pigs. *James E. Cook* 905

Infection in laboratory animals with pleuropneumonia-like organisms. *John B. Nelson* 911

Myocarditis and pulmonary arteritis associated with the presence of rickettsia-like bodies in polymorphonuclear leukocytes. *Alwin M. Pappenheimer* 921

Salmonellosis in laboratory animals. *Robert T. Habermann* and *Fletcher P. Williams, Jr.* 933

Bartonellosis. *Richard A. Griesemer* 949

Encephalitozoon infection in laboratory animals. *Donald H. Yost* 957

Joint diseases of laboratory animals. *Leon Sokoloff* 965

The identification and control of helminths in laboratory animals. *Robert T. Habermann* and *Fletcher P. Williams, Jr.* 979